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Factors Controlling the Input of Electrical Energy into a Fish (*Cyprinus carpio* L.) in an Electrical Field[1]

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ABSTRACT

The presence of a fish distorts the electrical field established initially in the water. This distortion, which changes the voltage gradient affecting the fish, and hence the electrical energy-input, is dependent upon the resistivity of the fish as compared to that of the surrounding water. These resistivities depend upon the temperature and chemical content of both water and fish. The criterion of electrical energy-input into the fish may be more important than voltage gradient in the water as an index to fish reactions to electrical fields. A method of measuring fish resistivity is described, and the results of an investigation of the effects of temperature and resistivity on water-to-fish resistivity ratio is presented. The idea is discussed that variation of these conditions may cause changes in energy-input to the fish by factors of ten or twenty even though voltage gradient in the fish-free water remains constant.

INTRODUCTION

When electrical methods are employed in the capture of fish it is found that voltages which have little effect on fish under some environmental and physiological circumstances may under other conditions have a much greater and perhaps lethal effect. Haskell (1954) has measured the snout-to-tail potential and resistivity of trout in various electrical environments, and Dengle (Denzer 1956) has measured resistances between various parts of a fish. Loeb (1955) has used Haskell's method in carp resistivity measurements. The purpose of this study is to investigate the factors governing the input of electrical energy into a fish while in an electrical field and held in a freshwater tank.

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In order to determine the electrical energy-input into a fish, both voltage and resistance, as applied to the fish itself, should be known. Neither of these quantities can be measured directly. The presence of a fish distorts the electrical field established initially in the water. This distortion, which changes the voltage gradient affecting the fish and hence the electrical energy-input, is dependent upon the resistivity of the fish as compared to that of the surrounding water. These resistivities depend upon the temperature and chemical content of both water and fish.

METHOD OF MEASURING FISH RESISTIVITIES

A tank made of plastic was found suitable for taking measurements of the resistivities of fish (*Cyprinus carpio* L.). When filled to the brim with water and no fish present, the resistance of the water in the plastic tank is

$$R_w = \rho_w \frac{S}{A} \qquad (1)$$

where R_w is the resistance of the water in ohms, ρ_w the resistivity of the water, S the length of the tank, and A the cross-sectional area of the tank. Measurements of the current and voltage show that in such a tank the field is uniform over the length of the tank, and that equipotential lines are at right angles to the long axis of the tank when the electrodes are at the ends of the tank. Precautions were taken to minimize the errors during measurement owing to electrolysis at the electrodes, and the setting up of reverse or counter EMF's. Frequent reversal of the current helps to minimize such errors. Measurements were also made with a fish in the tank. The fish was carefully placed in the tank so that the water level was maintained at the brim. This technique allowed the volume relationships between the fish and the water to be determined readily. The computation of fish-resistivity was simplified by designing the tank so that its dimensions bore a definite relationship to the shape of the fish whose characteristics were to be measured.

It is assumed that the fish may be considered as a parallelepiped for purposes of electrical measurements and computations. The effective length of the fish is designated as sS and its effective cross-sectional area as aA, where s and a are the ratios between the dimensions of the fish and those of the tank. It is also assumed that when the fish is in the tank, the lines of current-flow are everywhere parallel to the long axis of the tank. In a small tank, especially one having a cross-sectional area not much larger than that of the fish, this assumption is acceptable.

The tank containing the fish may be considered to be composed of two resistances in series. The first resistance is a section of water only, of length $S - sS$ and cross-sectional area A, the second resistance consists of two resistances in parallel, the "parallelepiped" fish of length sS and cross-sectional area aA, and a section of water of length sS and cross-sectional area $A - aA$. When these expressions for dimensions are used and combined with the usual relationships governing resistances in series and parallel, the result is:

$$R_f = R_w \left[1 - s + \frac{s}{ka + 1 - a} \right] \qquad (2)$$

In Equation (2) R_w, a, and s, have already been defined. R_f is the resistance of the tank including the fish, and k is the ratio between the resistivities of the water and the fish, defined by:

$$k = \frac{\rho_w}{\rho_f} \qquad (3)$$

Defining a quantity b as:

$$b = \frac{R_f}{R_w} \qquad (4)$$

and performing the necessary algebra, k can be expressed as:

$$k = 1 + \frac{s}{a(s + b - 1)} - \frac{1}{a} \qquad (5)$$

This relationship may be simplified and made more useful experimentally if the dimensions of the tank are all made proportional to the "parallelepiped" fish, or, if $a = s^2$ and $v = s^3$ where v is the ratio of the volume of the fish to the total volume of the tank. The value of v, and therefore that of a and s, is easily determined experimentally by noting how far the water in the tank drops in level when the fish is removed. With this simplification, Equation (5) becomes:

$$k = 1 + \frac{1}{s(s + b - 1)} - \frac{1}{s^2} \qquad (6)$$

Equation (6) has been used in this study, s and b being easily determined experimentally. Small errors in measurement of either s or b cause much larger percentage variations in the computed values of k. Measurements were taken as carefully as possible and the results are considered as a general index of trends.

MEASUREMENTS OF RESISTIVITIES OF CARP

The measurements of fish-resistivities were made on Carp No. 1 in a plastic tank level full of water. The tank measured 8 cm wide by 15 cm high by 61 cm long. Upon removing Carp No. 1 the water level dropped from the full 15 cm height of the tank to 12.5 cm. The volume-ratio, therefore,

was 2.5/15 or 0.167. R_w was 1800 ohms and R_f 1400 ohms, yielding for the ratio b the value 0.775. Using Equation (6) the resistivity ratio k was found to be 3. In other words, the resistivity of the body of the fish was about one-third that of the water in which it was immersed. The resistivity of the water, computed from the value of R_w and the dimensions of the tank, was found to be very nearly 3560 ohm-cm, the resistivity of the body of the live specimen was therefore about 1180 ohm-cm at 20°C. A day later measurements were made at 20°C with the same fish which was killed and then preserved by refrigeration overnight to determine whether or not the resistivity of a recently dead fish is materially different from that of a live fish. Previous measurements had been taken with the fish stunned by a blow, but still alive. These measurements, in summary, were: $R_w = 1950$ ohms, $R_f = 1550$ ohms, $k = 3$, $\rho_w = 3810$ ohm-cm, and therefore $\rho_f = 1270$ ohm-cm. This difference in the measurements of fish-resistivity, 1180 as compared to 1270 ohm-cm is not significant in view of the possible experimental errors. It appears reasonable to conclude that there is no material difference between the resistivity of the body of a live fish and that of a well-preserved recently-killed fish. When salt was added to the water in the tank until the resistivity of the water equalled that computed for the fish, no measurable difference in overall resistance was detectable when the fish was placed in the tank. It appears, therefore, that our method for measuring fish resistivity is substantially dependable.

EFFECTS OF TEMPERATURE ON RESISTIVITY

Measurements made on another carp (No. 2) to ascertain the variation of fish-resistivity with temperature gave the following results:

Temperature in degrees Centigrade:	5	10	15	20	25
Fish resistivity in ohm-cm:	2690	1840	1400	975	508

The resistivity of another carp (No. 3) was found to be 2800 ohm-cm at 5°C. Values for all three specimens are in good agreement. In taking measurements in water of low chemical content, it should be noted that the initially high water resistivity is decreased in a few minutes after the introduction of a fish owing to the production of mucus.

The energy-input into a fish varies inversely as the resistance of the fish, other variables being constant. Thus the energy-input could vary by a factor of about 5 times over the temperature interval between 5° and 25°C. However, other variables are probably not constant, and the energy-ratio can be much greater than 5 times, as is evident from the following analysis.

MODIFICATION OF ELECTRICAL FIELD IN THE VICINITY OF A FISH

A distortion of the normal electrical field in water occurs whenever the resistivity of the immersed fish is different from that of the surrounding water. The voltage gradient along the fish and the voltage from snout to tail are altered by this distortion, and thus are difficult to predict from measurements of voltage gradient taken in the water at even a short distance away from the fish.

For an investigation of the nature of this distortion a glass tank 117 cm long, 45 cm wide and with a water depth of 25 cm was used. The top of the tank was fitted with a sliding board extending across the tank. This board could be moved along the length of the tank. Mounted on the board was a block from which an exploring probe extended into the water. This probe was insulated except at the tip. A second probe was attached by soft wax to any desired point on the wall of the tank. The voltage at any point in the tank could therefore be determined with reference to the second probe. A high-impedance vacuum-tube voltmeter was used for measuring differences in potential so that the electrical field would not be distorted during measurement.

A double probe which could be revolved was also used and mounted above the tank on the sliding board. The two wire electrodes of the double probe were fixed approximately 1 cm apart. The direction of

the maximum potential gradient, which could be determined to within about 5°, was found, as expected, to be at right angles to the equipotential lines as determined by the single probe. The two measurements complemented each other.

Measurements of the distortion of the electrical field were made on Carp No. 1, which at this time was stunned but alive. Differences in voltage were measured over distances of both 33 cm and 25 cm in the tank before the fish was introduced. These distances were chosen because the length of the carp from snout to the base of the peduncle was about 33 cm, whereas the greatest body depth extended 25 cm back from the snout. Both fish and water were at 21°C at the time of the test. Before the fish was placed in the tank there was a uniform potential gradient of 0.22 volts per cm: over a 25-cm distance the difference was 5.7 volts, and over the 33-cm distance it was 7.4 volts. When the fish was placed in the tank, voltage differences near the fish immediately changed. Over the 25-cm anterior end of the fish the voltage difference changed to 4.6, and over the 33-cm distance it changed to 6.4 volts. These voltage differences are 83 per cent and 86 per cent of the values measured for these distances before the fish was introduced.

The fish-resistivity was in this case about one-third that of the water. It is reasonable to assume that whenever the resistivities are in this proportion a fish will be subjected to about 85 per cent of the voltage gradient as measured in fish-free water. Other percentages should be associated with other resistivity ratios. Further investigation can determine corrections to be made under varying circumstances. Readings taken on the dead carp after refrigeration overnight gave essentially the same results and were consistent with measurements taken previously.

Equipotential contour-lines of the electrical field around this fish were determined along with potential gradients as measured with the double probe. Results are shown in Figure 1. The distortion of the field is particularly noticeable at the anterior head-region. Whereas the potential drop per unit length along the body of the fish is less than in the fish-free water, the potential drop per unit length in the water immediately anterior to the tip of the snout is greater than before.

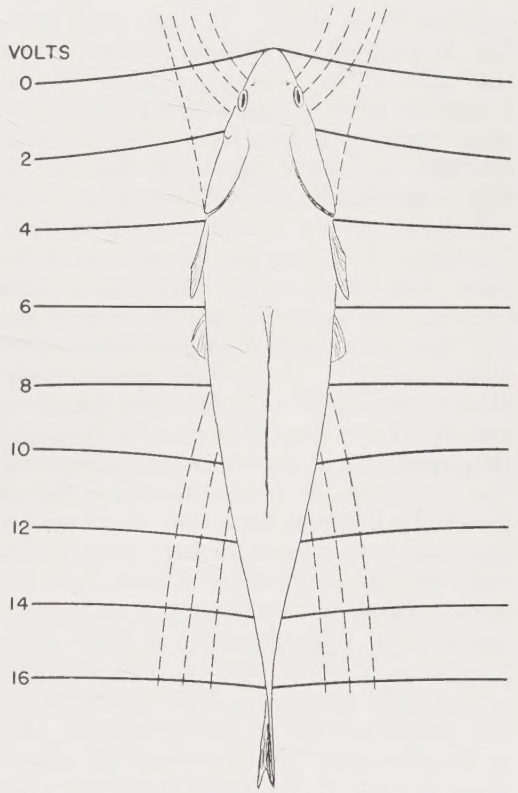

FIG. 1. Top view of fish in water showing moderate distortion of electric field by fish. Solid lines are lines of equal potential, and dashed lines show direction of current flow. Water resistivity was 3800 ohm-cm and fish resistivity 1270 ohm-cm, both at 20°C. Water-to-fish resistivity ratio was approximately 3.

Contour lines were also determined for another carp after the water had been made sufficiently saline to make its resistivity equal to that computed for the fish. In this case it was found that the contour lines as expected were at right angles to the length of the tank and not at all different from what they were without the fish.

EFFECT OF LOW CONDUCTIVITY WATER

Measurements were made with the fish in water of high electrical resistivity (low salt content and low conductivity). A condition similar to this would exist in

water containing few dissolved salts, a situation which occurs frequently in nature. To obtain low conductivity water 136 kilograms of clear ice were melted in the large tank. The first resistivity measurements on this water, when mixed with small pieces of ice and averaging 1°C was about 46,000 ohm-cm. The resistivity dropped to about 25,400 ohm-cm at 5°C. Since time elapsed between these measurements, substances may have entered the water from the glass walls, although the tank had been rinsed.

On the basis of the results previously described it was realized that the introduction of a fish into this water would result in a lowering of its resistivity. Carp No. 3 (dead) was therefore carefully washed off to eliminate excess mucus before being introduced into the tank. In spite of this treatment, however, it was found that the resistivity of the water, owing to mucous and other exudates from the fish, dropped in a short time from 25,400 ohm-cm to about 19,000 ohm-cm. The water resistivity was still high, however, and interesting results were obtained.

Before the carp was introduced, the potential gradient throughout the tank was 0.63 volts per cm, after the fish was introduced the potential gradient along the fish from snout to a point 25 cm posterior dropped to 0.26 volts per cm! This was only about 41 per cent of that measured without the fish. Even on the wall of the tank opposite the fish the gradient was only 0.435 volts per cm. In other words the total area or volume of distorted field is considerable, especially when the ratio of resistivities is large. In this case the ratio of resistivities of water as compared to the fish was approximately 7 or 7.5 to 1. This resistivity ratio is probably to be associated with a reduction of the electrical field to about 41 per cent over the mid-body region of any fish, as compared to the electrical field as measured in water without a fish.

TOTAL EFFECT ON ELECTRICAL ENERGY-INPUT INTO A FISH

The rate of electrical energy-input into a fish can be expressed by the relationship:

$$P = \frac{V^2}{R} \tag{7}$$

where P is the power input in watts, V the voltage effective over the length of the fish, and R the effective resistance of the fish.

Suppose a fish migrated from cold water of high resistivity to warm water of low resistivity similar to the situation of this study. Assume the same potential gradient was maintained in both the fish-free cold and warm water. In migrating from cold to warm water the potential difference from the snout to tail would change from about 41 per cent to about 83 per cent of the measurements taken in fish-free water, or in other words the voltages would about double. And since the power varies as the square of the voltage the energy-input would increase by a factor of 4. When allowance is also made for the change in resistance of the fish's body owing to change in temperature, which for a change in temperature from 5°C to 20°C is about 2½ times, the overall change in energy is ten to one. Had the temperature changed to 25°C the overall increase in energy would be closer to twenty to one.

It is evident that a potential gradient having little or no effect on a fish in cold water might be enough to kill the same fish in warm water. Such contrasting effects have been observed in the field and have been reported by Erkkila *et al.* (1956), who noted that game fish were unaffected in cold months, but when subjected to apparently the same potential gradients in summer were killed.

Figure 2 shows the potential lines around a fish when immersed in high-resistivity water. The distortion of the electric field is greater than in water of low resistivity. As compared to undisturbed water, the potential gradient along the fish is much less than before, but the potential gradient immediately ahead of the snout of the fish is greater than before. A small fish entering this region should be in greater danger because of the presence of the larger fish.

Another point should be noted. Fish used in these tests were never longer than

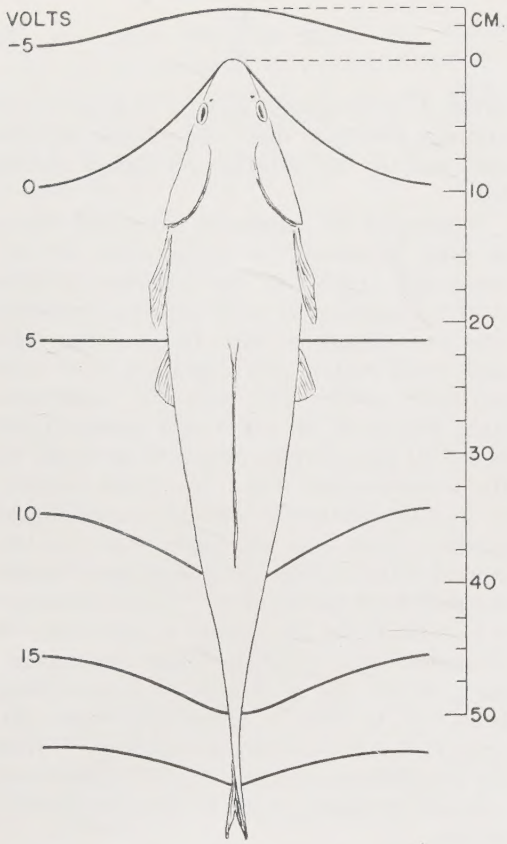

FIG. 2. Increased distortion of electric field as a result of increased water-to-fish resistivity ratio. Water resistivity was 19,000 ohm-cm, fish resistivity 2700 ohm-cm, and temperature 5°C. Water-to-fish resistivity ratio was approximately 7.

about one-third the total length of the tank. If a fish had been used equal in length to the tank, the total potential drop over its body should have been essentially equal to the drop between the electrodes. Possibly a situation similar to this may exist under field conditions. A long fish, or one nearly equal in length to the distance between electrodes, would be subjected to a greater potential gradient than a shorter fish. This factor would tend to discriminate against the longer fish.

Further work should be done to investigate the resistivities of different kinds of fish and the variation of these resistivities with temperature. Correlations should be made between these resistivities, the resistivities of water under various conditions, and the resulting modifications of the gradients of the electrical field. This information is needed before relative electrical energies can be found. It may be that the resistivities of most fish are about the same, if not, differences might prove useful in electrical techniques used in capturing or repelling fish.

SUMMARY

In the application of electrical methods to the control of fish it may be possible that one of the important factors which should be known is the electrical energy-input into the fish. This electrical energy is dependent upon the voltage gradient existing in the fish and upon the resistivity of the fish. The voltage gradient along the fish is not the same as that measured in the water, since the pattern of the voltage gradient existing in the water is modified by the presence of the fish. The lower the fish's resistivity as compared to that of the water the greater the modification of the electrical field and the smaller the voltage gradient existing in the fish. The resistivity of water changes with its chemical composition. The resistivities of both water and fish change with temperature.

It has been found that under the same conditions of voltage gradient existing in water the energy-input into a fish may differ by a factor of as much as ten or even twenty times, depending on temperature and chemical content of the water. Such differences are often encountered under natural conditions. Early in the season the water may be cold and relatively salt-free as a result of melting snows, whereas later in the season at the same location the water may be warmer and with more salts in solution. Under the former conditions a fish is doubly protected against electrical damage: his body has a higher electrical resistance, and, due to the still higher relative resistance of the surrounding water, he is subjected to a substantially reduced voltage owing to the increased distortion of the electric field under these conditions.

His rate of electrical energy-input, V^2/R, may therefore be only from one-tenth to one-twentieth as much as that under warmer water conditions.

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Life History of a Dinoflagellate Bloom[1]

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ABSTRACT

A bloom of *Gymnodinium* sp. is described in terms of its distribution, growth rate, production, and rate of diffusion. Production measurements on a second bloom of the same organism are also cited. In the center of the first bloom cell concentrations ranged from 1 to 18 million cells per liter. Net production ranged from 2.18 to 13.7 mg C/liter/day. Coefficients of horizontal eddy diffusion as calculated from the observed dinoflagellate distributions were low, ranging from near zero to 350 g/cm/sec.

INTRODUCTION

In late February and early March of 1955 a small but dense concentration of a dinoflagellate of the genus *Gymnodinium* was observed in the extreme upper end of the Duplin River at Sapelo Island, Georgia. This river is estuarine in character and receives practically no freshwater run-off from the land. Hence there is no systematic flushing action. The average depth of the water at mean low tide ranges from one meter in the region of the center of the bloom to 2 to 3 meters at the downstream edge. The bloom was observed to increase rapidly in total biomass and then decrease and disappear, but its center of distribution showed no horizontal movement. Remaining stationary and being restricted in size, the bloom presented an excellent opportunity for detailed observation of its development and for measurements of its productivity.

Another less dense bloom of the same organism was observed in the same location in May 1956. Discussion of the 1956 bloom will be restricted to the section on production. All other data and discussion will refer to the 1955 Bloom only.

METHODS

Six sampling stations were chosen starting at the center of the bloom and extending downstream (Fig. 1). Samples were dipped from the upper 10-cm layer along a transect across the river at each station and pooled to obtain a single composite sample. By this procedure variations due to local concentrations caused by wind drift and eddies were largely eliminated. Samples were collected each day at the same tide stage (near low slack water) as indicated by a staff gauge at Station 3. By using two outboard motor boats it was possible to collect composite samples at all six stations within a time interval of 15 to 25 minutes each day. For the 4–7 March surveys sampling was carried out between 1055 and 1504 E.S.T., according to the time of slack low water.

Dinoflagellate cells were counted after filtering an aliquot through an AA Millipore Filter. For a more complete description of this method see Pomeroy, Haskin, and Ragotzkie (1956).

Transparency of the water was measured by an instrument consisting of a self-contained light source (an ordinary 4.5-volt flashlight bulb) and a hermetically sealed Weston photocell. The light path was 18.7 cm, and extraneous light was excluded by means of a collimator (Williams 1953) built into the instrument. This instrument was designed and built by Dr. Lester V. Whitney.

DISTRIBUTION

The bloom occurred in the uppermost 2 kilometers of the river, which has a total length of 11 kilometers. The river receives

[1] Contribution No. 5 from the Marine Biology Laboratory, University of Georgia, Sapelo Island, Georgia. This research was supported by funds from the Georgia Agricultural and Forestry Research Foundation.

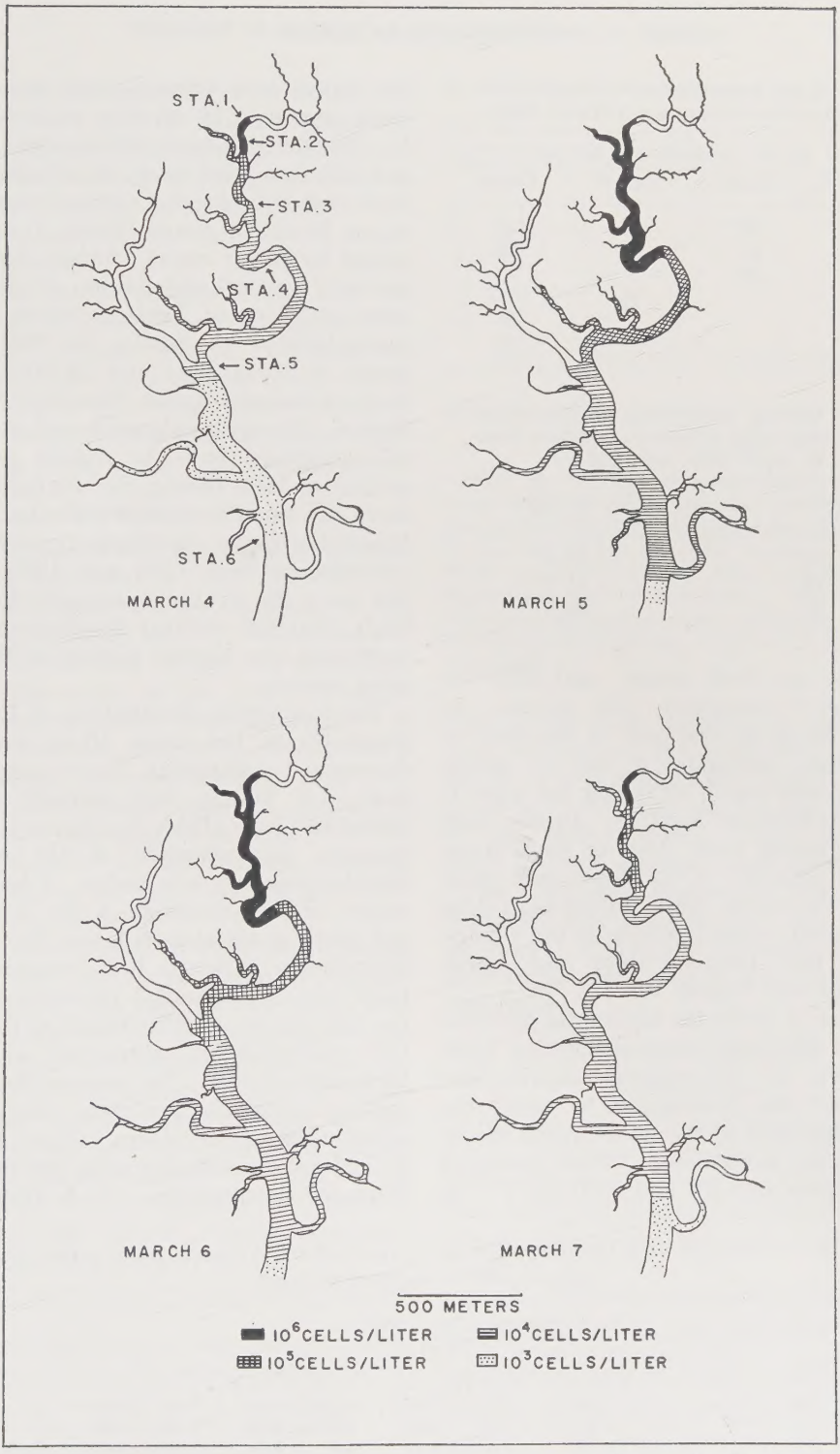

FIG. 1. Distributions of *Gymnodinium* sp. in the upper 10-cm stratum of the Duplin River for 4–7 March 1955. The map of the river was drawn from an aerial photo mosaic made from official U. S. Navy aerial photographs. Both the mosaic and the photographs were supplied by the Glynco Naval Air Station, Brunswick, Georgia.

TABLE 1. *Light transmission in Duplin River in dinoflagellate bloom on 1–3 March 1955*

Depth cm	Per cent transmission over light path of 18.7 cm		
	1 March	2 March	3 March
2	13	15	21
10	20	20	26
20	20		
30		21	
50			27
100			27

TABLE 2. *Vertical distribution of* Gymnodinium *sp. in a small tidal tributary of Dublin River, 21 April 1955, 1030 E.S.T.*

Depth cm	No. cells /liter $\times 10^6$
1–2	45.6
10	41.2
20	20.8

essentially no fresh water, and thus its circulation is completely tide driven. As the tide range on this part of the coast is fairly large, averaging 8 feet at spring tides, the salt marsh adjoining the river is subject to frequent flooding. During high water of spring tides three or more times the tidal capacity of the river itself spills out over the marsh, while during neap tides there is nearly equal partition of the volume of total flow between river and marsh (Ragotzkie and Bryson 1955).

In order to study the horizontal distribution of a plankton bloom something must be known of its vertical distribution. This factor was investigated by measuring the transparency of the water in the bloom area with the photometer at 10-cm intervals of depth between 1000 and 1200 E.S.T. In the bloom area transparency was consistently greater at 10 cm than at 2 cm (Table 1). In another bloom of this same organism in a different tidal creek, the dinoflagellates were found to be most concentrated in the upper 10 cm of water (Table 2) as determined by direct count. Other direct estimates of vertical distribution of dinoflagellates are given in Table 5, where the cell concentrations in bottles for the experiments of 20–21 May and 29–30 May are those of samples drawn from the indicated depths. From these data it would seem that this organism tends to remain near the surface at least during the daylight hours. Since the samples collected during the 4–7 March horizontal distribution surveys were collected between 1100 and 1500 E.S.T., and since the weather remained clear, it is likely that the vertical distribution of the organisms was similar during all the sampling periods.

The horizontal distribution of the dinoflagellates in the upper 10-cm layer was determined completely for 4 consecutive days, 4–7 March, and partially for two additional days (Table 3). Figure 1 shows a synoptic representation of the complete distributions at low water. Clearly the center of concentration of the bloom did not move systematically either upstream or downstream, although it is recognized that there was a horizontal oscillation due to the tidal action. In the region of the bloom this oscillation was estimated to be one kilometer or less. The present description applies only to the low water position and distribution of the bloom. From 4 March to 5 March the concentration contours were displaced downstream. On 6 March little

TABLE 3. *Concentrations of* Gymnodinium *sp. in upper 10-cm stratum of water, expressed as 10^6 cells per liter ± standard deviation*

Date	Station					
	1	2	3	4	5	6
3 March	3.0 ± 0.34	2.3 ± 0.17	0.15 ± 0.029	0.16 ± 0.052	0.062 ± 0.022	
4 March	5.5 ± 0.57	3.3 ± 0.64	0.12 ± 0.042	0.027 ± 0.034	0.015 ± 0.034	0.001*
5 March	2.7 ± 0.77	18.7 ± 5.1	4.3 ± 0.65	1.8 ± 0.44	0.10 ± 0.02	0.027 ± 0.010
6 March	3.4 ± 1.7	4.4 ± 3.3	1.1 ± 0.35	1.4 ± 0.22	0.29 ± 0.055	0.028 ± 0.016
7 March	2.1 ± 0.93	1.5 ± 0.34	0.82 ± 1.0	0.054 ± 0.033	0.035 ± 0.013	0.012 ± 0.014
10 March	0.33 ± 0.057	0.16 ± 0.040	0.038 ± 0.017			

* Too few cells counted.

change was noticeable, but by 7 March the contours were located further upstream. The bloom was first observed visually on 28 February, and during the next few days it gradually increased in size. However the maximum concentration remained in a very restricted area (Fig. 1). After 7 March the bloom was no longer visible and was presumed to have decreased sharply in biomass. This was verified by a sampling survey of the center of the bloom on 10 March (Table 3). From this sequence it appears fairly certain that the distribution study was made at the height of the bloom.

GROWTH STUDIES

Growth rate of the *Gymnodinium* population was estimated by two methods: (1) direct estimation of the changes in the entire population, and (2) bottle experiments. In the first method the number of cells in the upper 10 cm over the entire bloom was estimated from the product of the area between successive concentration contours and the mean population density per unit area between contours. Summing these products then gives an estimate of the total population in the stratum sampled. The results for the 4-day distribution study are given in Table 4.

In the second method a suspension of the cells in river water was placed in a gallon jug. The jug was exposed to sunlight and kept in a tub of water for temperature control. The concentration of cells was determined at hourly intervals by the Millipore Filter method. The number of cells counted ranged from 729 in the first sample to 1126 in the last sample. A linear regression of the ln concentration on time was calculated, and fiducial limits determined for the resulting slope (Fig. 2).

The jug experiment was done on 4 March,

TABLE 4. *Estimates of the total number of cells in the upper 10 cm of the entire bloom*

Date	No. of cells $\times 10^{11}$
4 March	20.8
5 March	242.
6 March	113.
7 March	17.6

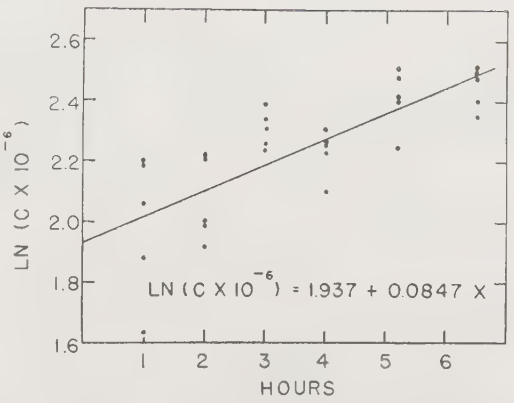

FIG. 2. Growth-rate experiment. Ln concentrations of dinoflagellates in jug plotted against time showing the linear regression of best fit as calculated by least squares. Slope = 0.0847 ± 0.0270 at the 95% confidence level. Generation time = 11.8 hours. The confidence limits for generation time calculated from the 95% limits of the slope are: l_1 = 8.9 hours, l_2 = 17.3 hours.

the first day of the distribution study. The best estimate of the generation time obtained by this method was 11.8 hours compared to 7.1 hours for the 4–5 March change in the entire bloom. These generation times are not strictly comparable because in the direct field method net changes were measured over a whole day, half of which was dark. Also in the field study there may have been some losses to the marsh during high tides.

PRODUCTION

1955 bloom

Rapid growth of a phytoplankton population is in itself an indication of high production. Direct measurements of production were made by determining changes in dissolved oxygen in light and dark bottles (Table 5).

In the 1955 experiments dissolved oxygen was determined by the unmodified Winkler method while in 1956 both the unmodified Winkler method and the Pomeroy-Kirschman-Alsterberg sodium azide modification (APHA 1955) were used in order to determine the effects of high concentrations of organic matter and supersaturation of oxygen. The sodium azide modification gave slightly higher dissolved oxygen values

TABLE 5. *Results of production measurements for dinoflagellate blooms*

In the 1–2 March 1955 experiment a suspension of dinoflagellates collected from the upper 10 cm was incubated at other depths as indicated. Several measurements made under "no bloom" conditions are included in summary form for comparison. All measurements were made over a 24-hour period unless otherwise noted.

Date	Station	Depth m	Cell concentration in bottles $\times 10^6$	Extinction coefficient per meter	Oxygen changes (ml/L)		Mg C fixed/liter/day	
					light bottle	dark bottle	Net	Total
1–2 Mar '55	5	0.35	26	1.15	25.5	−5.13	13.7	16.5
		0.85	26		21.7		11.7	14.8
		1.85	26		6.52		3.51	6.27
		2.85	26		−1.13		0.61	2.19
		3.85	26		−3.78	−5.20	−2.03	0.76
2 Mar '55	exposed to direct sunlight for 2 hrs.		4.6		6.45/2 hrs.			
20–21 May '56	1	0.05	0.68	2.35	8.79 (3)*	−2.10 (3)*	4.71	5.84
		0.50	0.92		5.10 (2)*	−1.80 (1)*	2.74	3.70
		1.00	0.16		2.32 (2)*	−0.98 (1)*	1.24	1.77
29–30 May '56	2	0.05	0.20	3.45	4.06 (4)	−2.50 (4)	2.18	3.51
		0.05	0.20		4.19 (4)*	−2.41 (4)*	2.25	3.54
		0.50	0.24		2.17 (2)*	−1.98 (2)*	1.16	2.22
		1.00	0.07		0.96 (2)*	−2.10 (2)*	0.52	1.64
30–31 Mar '55	5	0.5	no bloom				0.27	—
5–6 Apr '55	5	0.5	no bloom				0.18	0.32
4–5 May '55	5	0.5	no bloom				0.46	0.65
18–19 May '55	5	0.5	no bloom				−0.10	0.45
13–14 Jun '55	5	0.5	no bloom				0.37	0.56

( ) Number of replicate bottles.
* Sodium azide modification. All other O_2 determinations by unmodified Winkler Method.

(Table 5); therefore, the production values for the 1955 experiments are probably slightly low.

Water from the upper 10 cm in the center of the 1955 bloom was put in citrate bottles and incubated for 24 hours at various depths from 0.35 to 3.85 m at a point several hundred meters downstream. The cell concentration was 26 million cells/liter. Although this procedure was somewhat artificial, the results are included in Table 5 to show the rate of production under various light conditions. At the 0.35 m level a net production rate of 13.7 mg C/liter/day was obtained, and it is reasonable to suppose that production within the bloom was the same order of magnitude.

A second estimate of production in the bloom was made by placing a suspension of cells in citrate bottles (6 cm in diameter x 12 cm high) and exposing these to direct sunlight from 1100 to 1300 E.S.T. The concentration used was 4.6 million cells/liter. The bottles were kept cool by placing them in a tub and barely covering them with river water. This procedure was more realistic in that the cells received an amount of light similar to those in the bloom. Net production in these bottles was 1.73 mg C/liter/hour.

1956 bloom

Two production measurements on the 1956 bloom were made by incubating suspensions of the dinoflagellates at the station and depth at which they were collected. Bottles were incubated at 0.05, 0.5, and 1.0 m depth, the total depth at this station at low tide being slightly more than 1 m. This presumably was a closer approach to the natural situation than the 1955 experiments. Net production at the 0.05 m level by cell concentrations of 0.68 and 0.20 million cells/liter was 4.7 and 2.2 mg C/liter/day, respectively. By plotting the observed changes in oxygen concentration against depth and integrating the area under the curve, the net production on an areal basis was found to be 0.287 and 0.130 mg C/cm^2/day.

Comparison with other production measurements

In the same region of the Duplin River measurements of net production in the spring season when no bloom was present gave values ranging from −0.011 to +0.059 mg C/cm²/day. On a volume basis the net production at 0.5 m varied from −0.105 to +0.465 mg C/liter/day. Clearly production during the dinoflagellate blooms was one or more orders of magnitude greater than when no bloom was present.

Conover (1954), measuring production by dinoflagellates in two blooms in New Haven Harbor, obtained net changes in oxygen at the surface of 1.53 and 1.58 ml O_2/liter/day. In terms of carbon fixation these values are 0.82 and 0.85 mg C/liter/day. The dinoflagellate concentrations were 44.4 and 6.2 million cells/liter respectively.

Total radiation values for the dates of the Sapelo Island blooms as estimated by the method of Kennedy (1949) were 487 g-cal/cm²/day on 1–2 March and averaged 703 g-cal/cm²/day in the period 20–30 May, while for New Haven Harbor the values were estimated to be 728 and 707 g-cal/cm²/day on 24 June and 7 July. Thus the New Haven Harbor blooms received more radiation than those at Sapelo Island. Except for the fact that different genera were involved in the two places, no explanation for this large difference in production values can be offered.

DIFFUSION

Starting at the mouth of the Duplin River the first tidal prism extends nearly 5 kilometers upstream (Ragotzkie and Bryson 1955). Because of the extensive flooding of the marsh the volume of water involved in the tidal pulse decreases sharply upstream, and consequently the length of the upstream tidal prisms most likely also decreases. Therefore, as there is no flushing by fresh water and no permanent stratification of the river, exchange between the upper reaches of the river and the mouth of the river must be accomplished primarily by diffusion. The coefficients of horizontal eddy diffusion for the upper 10 cm of water in the bloom area can be computed from the distributions of organisms over several consecutive days.

Starting with the equation for the distribution of a non-conservative variable in the sea which takes into account the effects of advection, diffusion, and biological change (Sverdrup *et al.* 1942) the following simplifications were made. The longitudinal axis of the river was considered as the x-axis, and the y-axis was taken cross river. As already pointed out, the center of the bloom did not move systematically either upstream or downstream. Therefore advection in the x direction was zero and can be ignored. By collecting composite samples at each station both advection and diffusion in the y direction were eliminated. Since the organisms in this bloom appeared to be positively phototactic, the vertical terms also became negligible. By making these simplifying assumptions the equation is reduced to:

$$\frac{\partial s}{\partial t} = \frac{\partial}{\partial x}\left(\frac{A_x}{\rho} \cdot \frac{\partial s}{\partial x}\right) + R$$

where s = concentration of the dinoflagellates

A_x = coefficient of eddy diffusivity for the longitudinal axis of the river

R = biological change (net effect of reproduction and mortality).

In the section on growth it was shown how the total number of cells in the whole population each day was estimated from the field data. The day to day changes resulted from the combined effects of reproduction and mortality including losses to the marshes during high water. It was assumed that these changes were taking place uniformly throughout the entire bloom area. In the equation, R was assigned these values. Local changes, $\frac{\partial s}{\partial t}$, at each station for each pair of days were available directly from the sampling data. Mean horizontal concentration gradients for each day of a pair of days and in both upstream and downstream directions were obtained from the observed distributions. Eddy diffusion coefficients could now be

TABLE 6. *Coefficients of horizontal eddy diffusion (g/cm/sec) as determined from the distribution of dinoflagellates*

Station	4–5 March	5–6 March	6–7 March	Width of river m
2	9.0	1.8	−3.9	23
3	7.9	−2.3	72.	38
4	92.	72.	350.	54

computed on a finite basis using two tidal cycles or about 25 hours as the time interval. The results are given in Table 6. No values could be estimated for Stations 1 and 6 because horizontal concentration gradients were known in only one direction from these stations. Station 5 was omitted because it was located at a major fork in the river where diffusion could take place in three directions. The two negative values obtained were probably due to the failure of the assumption that R was everywhere the same. With the exception of these two values the trend of values is quite reasonable, showing that diffusion increases downstream as the river becomes wider. The values themselves are very low thus indicating a high degree of hydrographic isolation of this area.

It should be emphasized that these coefficients were determined over a time interval of two tidal cycles and therefore represented the net transport by unorganized motion of the water that had occurred during two rising and falling tides and included the effects of the great increase in surface area of the water brought about by the flooding of the marsh. Thus the effects of several different changing situations have all been combined under the head of diffusion.

The bloom described here fits quite well the linear case treated by Kierstead and Slobodkin (1953) in their analysis of the size of water masses containing plankton blooms. They postulated the equation

$$L_c = \pi \sqrt{\frac{D}{K}}$$

where L_c is the critical length of the water mass below which diffusion effects exceed growth, D is the diffusion, and K the increase in population. In the present case leakage by diffusion was probably occurring at only one end; therefore the diffusion term will be halved. Applying the equation to the data of Station 2 on 4–5 March when the bloom was still increasing, L_c comes out to be 16 meters. Clearly in the upper reaches of a tidal river such as this, diffusion is so small that a bloom could start from practically a point source and still maintain itself.

Actually when this bloom was first observed, it was distributed in several patches a meter or two in extent along a distance of 100 meters or less of river. Its subsequent development during which high concentrations were maintained in the source area demonstrates that in this restricted situation the critical size of the water mass was certainly small.

DISCUSSION

From the information presented here it is clear that this algal bloom resulted from an explosive growth of one species and not from any large-scale horizontal concentration effect. Vertical migration cannot be ruled out entirely, but the shallowness of the water (about 1 m in the center of the bloom at low tide) precludes concentration factors of more than 10, and the vertical distribution data for the 1956 bloom (Table 5) indicate that the maximum factor was probably nearer 2.

Diffusion effects tended to disperse the organisms, but because of the restricted nature of the area, diffusion was less than biological growth thus allowing concentrations to increase. Because the bloom was hydrographically isolated from other water masses and subjected to relatively low diffusion rates, it was possible to measure its growth directly. Immediately before the peak population was reached the organisms were dividing at a rate of about once every 7 to 12 hours.

Diffusion rates for the restricted upper end of a tidal river were found to be very low. Thus the area is nearly a closed system subject to tidal fluctuations but not systematically flushed as is an estuary. Transport of nutrients into this type area

would be rather slow, and similarly biologically produced metabolites would be removed at a low rate and would tend to accumulate at the site of production.

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Effects of Lake Fertilization by Volcanic Activity on Abundance of Salmon[1]

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ABSTRACT

Evidence from various sources—tree growth during the past century, chemical composition of waters of various lakes, plankton volumes, size of young salmon migrating seaward—tends to indicate that the fertilization of lake waters in western Alaska by volcanic ash during sporadic eruptions may be an important factor in determining the abundance of sockeye salmon.

This paper discusses the possible relation between volcanic activity and salmon production, especially of the sockeye or red salmon, *Oncorhynchus nerka*. The young sockeye usually remain in lakes until their second, third, or fourth year before migrating seaward to complete their growth, and thus the young of this species of salmon are most affected by any changes in the freshwater habitat, such as are wrought through volcanic activity.

Western Alaska (Fig. 1) has long been one of the richest fishing areas in the world; for amost fifty years the value of the annual salmon catch has averaged more than 30 million dollars by present-day standards. The abundance has been declining steadily, however, for several decades, until today in spite of fair numbers of spawners these fisheries are in critical condition. This area has also been noted for volcanic activity throughout historic time. That such activity was on a much larger scale prior to the advent of white men can be deduced from the profusion of volcanic cones almost everywhere—some dead, others somnolent but displaying occasional signs of life.

Alaskan volcanoes usually throw out pumice in the form of finely divided ash or porous rocks, rather than ejecting molten lava as do many volcanoes in other regions. The fine material is carried by the wind for varying distances (up to 300 miles in many cases) and laid down as a blanket of varying depth. This ash layer may be an important factor in salmon production.

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VOLCANISM IN THE PENINSULA AREA

There is every reason to believe that most of the watersheds in this general area have received volcanic deposits, and in many instances so recently that a residual fertility probably remains. The most prominent of the volcanoes, starting at the southern end of the map (Fig. 1) are Mt. Veniaminof, Mt. Peulik, Mt. Katmai (including Novarupta), Mt. Trident, Mt. St. Augustine, Iliamna Volcano and Redoubt Volcano (this latter just off the map to the north).

Mount Veniaminof undoubtedly dropped large amounts of ash on the nearby lake system of the Chignik River when it erupted violently in 1892. Moving northeastward, the large Mt. Peulik volcano straddles the land bridge between Ugashik and Becharof Lakes, which must have received a large proportion of its ash. Next we come to the recently active Katmai volcanic area which we will discuss last.

The beautiful cone of Mt. St. Augustine on an island in the mouth of Cook Inlet erupted in 1883 and depending on the wind direction probably showered ash either on Iliamna Lake to the west or the watersheds of the Kenai Peninsula to the east.

The Naknek and Kvichak districts have a number of sporadically active volcanoes, some with craters so huge, e.g. Redoubt and Iliamna, that their outfalls could have made

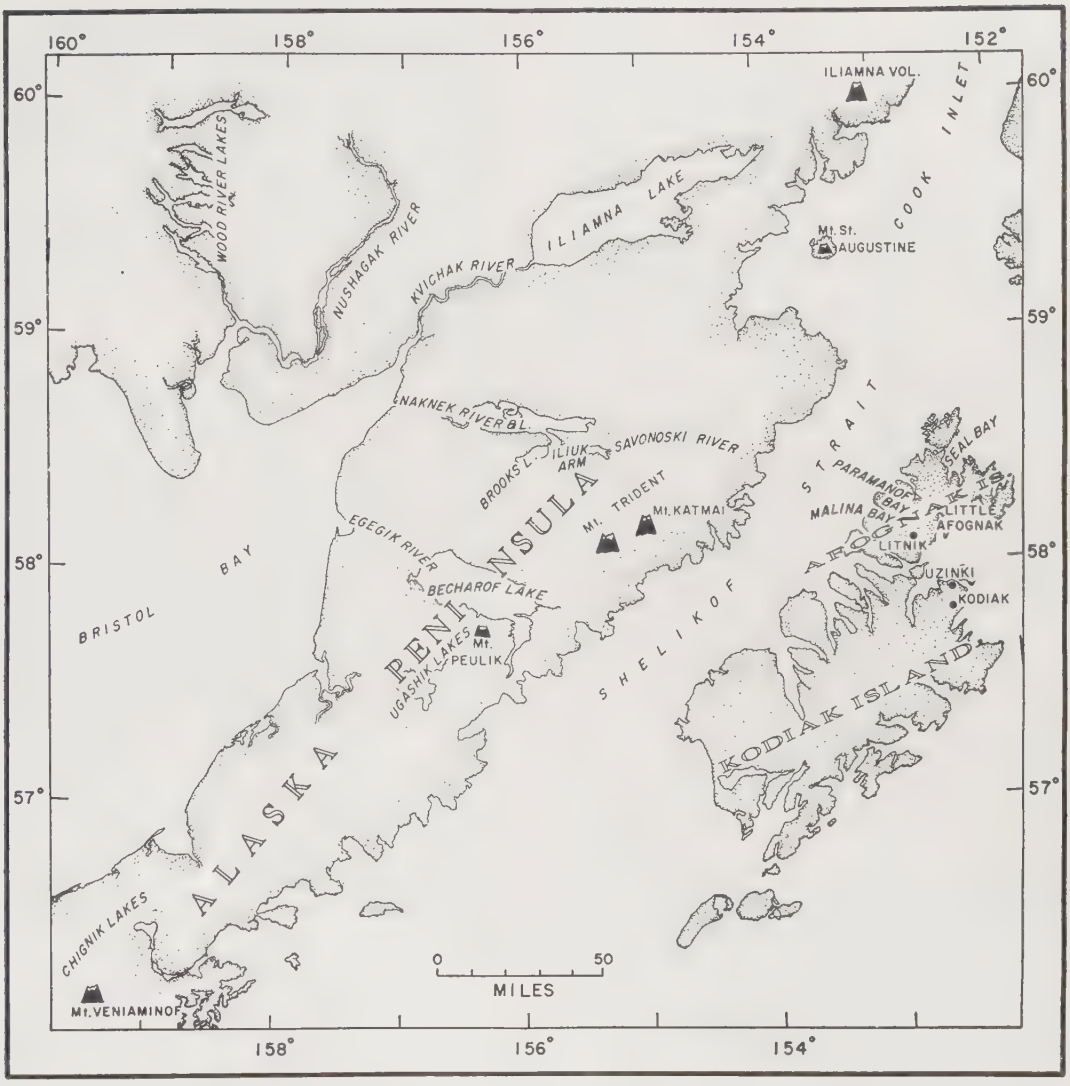

FIG. 1. Map of western Alaska showing location of places and volcanoes discussed in the text.

heavy deposits for at least a hundred miles in whatever direction the wind dictated.

Returning to the Katmai area, a great eruption occurred from June 6–12 in 1912 followed by a lighter eruption in September of 1913. Nearby Novarupta, a large but low volcano in an adjacent valley, threw out large quantities of ash and pumice at about the same time as the 1912 eruption of Mt. Katmai. Following a long period of dormancy Novarupta erupted violently for approximately two hours on May 19, 1949, a heavy outfall of ash being carried down Shelikof Strait by a westerly wind (observed by senior author). A few weeks later nearby Mt. Trident erupted, and it has exhibited sporadic activity almost to the present, although without significant ash outfall.

OBSERVED SHORT-TERM EFFECTS OF ASHFALL

Short-term effects of heavy ashfall on both fish and fish food organisms were observed during and following the heavy 1912

eruption of Mt. Katmai. Heavy clouds of ash were carried chiefly eastward by the wind prevailing at the time. According to Martin (1913) ashfall was between 10 and 20 inches in the area of Brooks Lake and Iliuk Arm of Naknek Lake but did not extend farther to the west. To the eastward the ash clouds were carried across Afognak and northern Kodiak Islands, the ash deposit reaching 10 inches at Kodiak and along Kupreanof Strait between the two islands.

Edward Ball (1914), inspector of Alaska salmon fisheries, stationed at Litnik on Afognak Island, gives a detailed account of the effect on salmon spawning and on fish food organisms. Since the eruption occurred before mid-June most of the salmon had not yet entered the streams, but these were suffocated by the turbid water and mud; approximately 4000 salmon died in Litnik Creek.

A survey of the principal stream systems on Afognak Island showed that in the eastern portion, where the ashfall was least, mollusks, worms, and some insect larvae could be collected. However, no crustaceans could be found. On the western side of the island the streams and lakes were almost destitute of fish food. The streams were so choked with sand and mud that about the middle of August several hundred salmon were suffocated in the same manner as just after the eruption.

During the following summer of 1913 attempts to collect seaward migrants (smolts) of sockeye salmon at Litnik Lake were made (Evermann 1914), but the number captured was so very meagre as to justify the conclusion that there had been a heavy loss of young salmon in the lake.

The question of the extent of this direct damage to the salmon runs and how quickly and how well the runs recovered can be at least partially answered by the records of the salmon fishery at three Afognak Island streams (Rich and Ball 1931). These give the annual catches by native fishermen for the canneries at Kodiak and Uzinki.

Data are available for most years from 1907 to 1921 (Table 1). Because the three streams are not of equal value as salmon producers, and because data are missing for some years prior to 1913, it has been necessary, in order to obtain the best estimate of relative abundance, to weight the catches for different streams. This weighting, based on the sum of the catches from 1913 to 1927 (except for 1916 when fishing in two of the streams was suspended), gives each stream equal weight and furthermore makes possible comparison of those years lacking data for one or two streams.

The average weighted catches and the trend smoothed once by a moving average of 3 are shown in Figure 2. The influence on survival of the heavy ashfall is very apparent in the small catches made from 1916 to 1920 when the returning adults would come from broods subjected to the ash while in fresh water. The young spend from 1 to 4 years (usually 2 to 3 growing seasons) in freshwater before descending to the sea. The majority of the adults are in their fifth year of life when they return from the sea on their spawning migration.

Thus the 1911 brood (run of 1916) would have just emerged from the spawning gravel at the time of the eruption in June 1912. The 1912 spawners were just commencing to ascend the streams. In 1913 the creeks were still ash-filled and a fresh eruption of smaller proportions occurred in September, 1913. The streams in 1914 were still very turbid following periods of rain.

The spawning runs from broods suffering major direct damage, especially the broods of 1911 to 1915 (returning as adults in 1916 to 1920), were small. However, the runs returning from the smaller numbers of spawners in 1916 to 1920 were fully as large as before the eruption.

The rapid recovery of these sockeye runs from the effects of the ashfall is of interest because when runs have been depleted by overfishing, lessening of the fishing intensity is seldom followed by such rapid recovery. This suggests very favorable environmental conditions, but whether these conditions were better food supply or perhaps a concomitant destruction of predators cannot be determined from the meagre data.

TABLE 1. *Sockeye salmon catches (in thousands of fish) for three streams on Afognak Island*

Year	Actual catches[1]			Catches weighted by factor			Average weighted catch	
	Seal Bay	Malina Bay	Paramanof Bay	Seal Bay	Malina Bay	Paramanof Bay	Average	Average smoothed by 3
1907	—	30	—	—	19.5	—	19.50	
08	16	7	—	24.5	4.6	—	14.55	23.53
09	21	63	—	32.1	41.0	—	36.55	26.37
1910	—	43	—	—	28.0	—	28.00	29.72
11	—	—	20	—	—	24.6	24.60	29.70
12	—	43	35	—	28.0	43.0	35.50	30.16
13	20	42	27	30.6	27.3	33.2	30.37	34.75
14	24	60	32	36.7	39.0	39.4	38.37	30.50
1915	18	42	11	27.5	27.3	13.5	22.77	27.28
16	7	(3)[2]	(2)[2]	10.7	—	—	10.70	14.99
17	7	12	13	10.7	7.8	16.0	11.50	12.61
18	7	14	22	10.7	9.1	27.1	15.63	15.35
19	12	23	19	18.4	15.0	23.4	18.93	16.82
1920	12	11	18	18.4	7.2	22.1	15.90	24.78
21	15	77	37	23.0	50.0	45.5	39.50	22.44
22	5	17	14	7.6	11.0	17.2	11.93	29.38
23	27	68	20	41.3	44.2	24.6	36.70	26.14
24	28	32	21	42.8	20.8	25.8	29.80	32.47
1925	27	28	27	41.3	18.2	33.2	30.90	33.24
26	20	101	17	30.6	65.6	20.9	39.03	26.77
27	8	12	9	12.2	7.8	11.1	10.37	
Sum 1913 to 1927 (Ex. 1916)	230	539	287					
Weight factor	1.53	0.65	1.23					

[1] Catches from Rich and Ball (1931).
[2] Fishing suspended after 3 weeks because of scarcity of fish.

FERTILIZATION OF WATERSHEDS BY ASHFALL

The evidence for significant fertilization of watersheds by ashfall and its long-term effect is based on plant and tree growth, on crude soil tests, and on the comparison of chemical content, plankton production, and smolt growth in lakes that received heavy ashfall from Katmai with lakes that did not.

Undoubtedly the chemical composition of the ash will vary in different eruptions and with distance and direction from the source. Griggs (1920) shows that the ash from the 1912 Katmai eruption included 0.36 per cent of phosphorus, 0.47 per cent of magnesium and 3.80 per cent of calcium. Phosphorus, especially, has been proved valuable in lake fertilization experiments. Unfortunately we have no phosphorus determinations for any of the lakes affected by the Katmai eruption.

The volcanic ash contains plant nutrients. At Kodiak where the ashfall averaged about ten inches, vegetation was greatly reduced during the first two years following the eruption due partially to smothering but perhaps also to overabundance of some chemicals and lack of humus. But Griggs (1920) found that after the second year, plant growth became so accelerated as to be far above normal.

The senior author has examined the growth history of five spruce trees, *Picea* sp., selected at random in 1951 from the forest near the shores of Brooks Lake, some 30 miles from Mt. Katmai. According to Martin (1913) this area received between 10 and 20 inches of ash deposit during the 1912 eruption, still clearly visible as a white pumice layer. Annual growth was determined by microscopic examination of radial cores removed from the trees with a

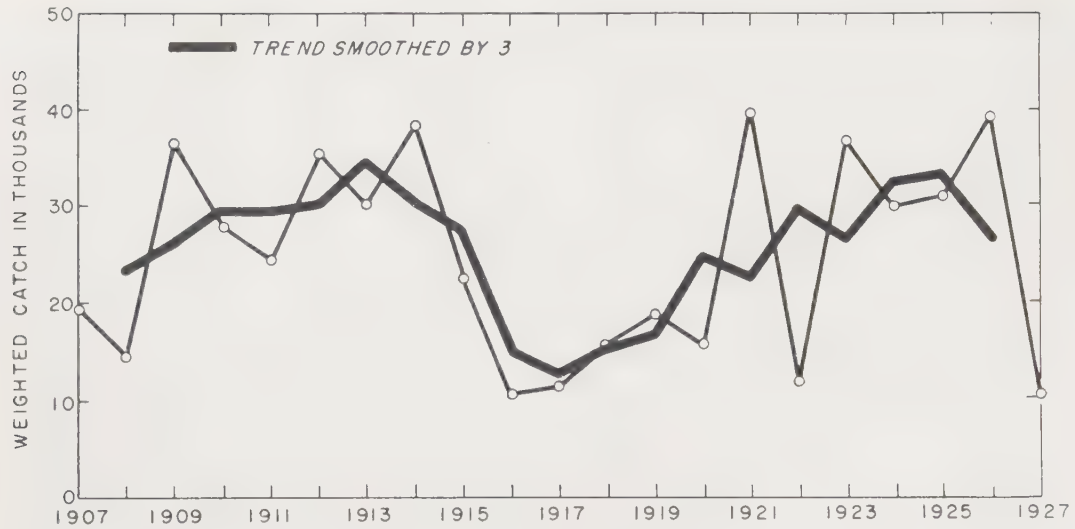

FIG. 2. Sockeye salmon catches from three areas on Afognak Island illustrating the short-term effect of the ashfall from the Katmai eruption of 1912 on salmon abundance.

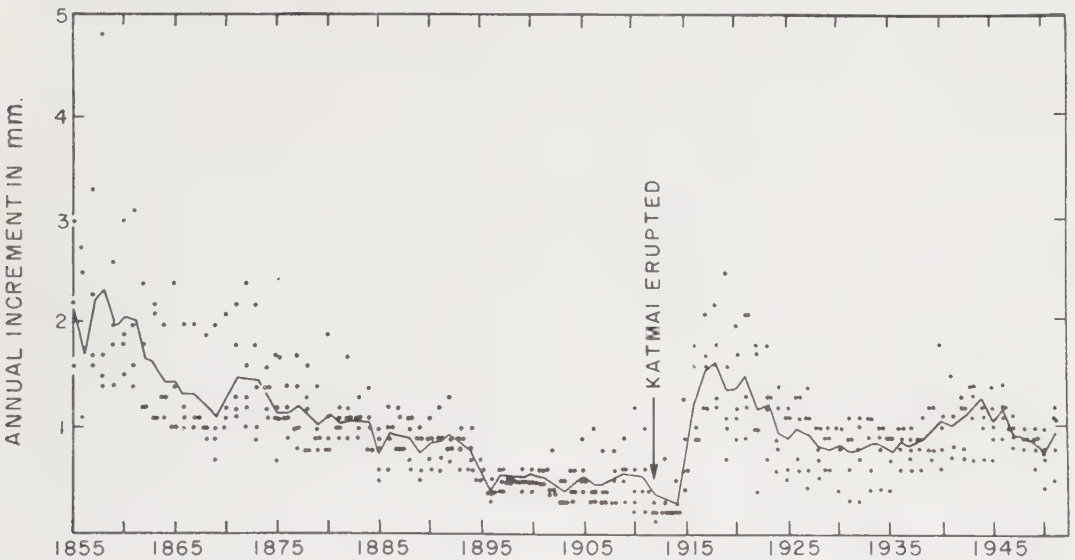

FIG. 3. Annual growth increments of five spruce trees from Brooks Lake, 1855 to 1951, illustrating the marked long-term effect of ashfall on soil fertility.

standard increment borer, utilizing a stage micrometer to measure the distance between adjacent growth rings.

Figure 3 shows the growth of these five trees from 1855 to 1951 when the cores were taken. The normal decline in growth rate with advancing age is clearly evident from 1855 until 1914, two years after the ash deposit from Katmai. After 1914 an abrupt and rapid upturn in growth occurred, reaching a peak in 1918. Growth has continued at a high level since that time. This parallels the observations of Griggs (1920) that two years of depressed growth preceded the accelerated growth of Kodiak vegetation after the eruption.

Enrichment of the soil by ash is corroborated by crude tests for nitrogen made by the senior author in 1947 at Brooks Lake. The top layer of soil, about an inch

or less in depth, consisted largely of dead and partly-decayed vegetable matter showing a slight nitrogen content. Immediately below this the white pumice layer 6 to 15 inches thick from the Katmai eruption was high in nitrogen. Below this lay a black stratum of fine, sandy loam two or three feet deep showing no measurable nitrogen reaction. Gravel and sand extended downward from this point. A westerly wind prevailed at the time of the eruption so that on the Alaskan Peninsula (Martin *loc. cit.*) only Brooks Lake and Iliuk Arm of Naknek Lake received significant portions of the ashfall occurring between June 6 and 12 of 1912. Over the years a large proportion of the materials of value to fish have undoubtedly been slowly leached from the soils, and either have become adsorbed in the bottom sediments or been lost from the freshwater cycle by being flushed out of the lakes in the outlet streams.

Since algae, at the base of the aquatic food chain, can readily utilize inorganic nutrients, they would be expected to benefit from chemical enrichment of the soils of the watershed by ashfall, and this increase in basic food should be reflected in better growth rates of the young sockeye salmon which are pelagic in the lakes.

As mentioned above the young remain from one to four years in nursery lakes before dropping down to the sea for completion of their growth. The rates of survival of young red salmon in a lacustrine environment depend to a large extent on the rates of growth, since the faster they grow, the fewer are taken by predators. Ocean survival has also been shown by Kelez (1937), Barnaby (1944), and Foerster (1954) to depend to a large extent on the size of the fish when they reach the sea, the larger smolts having enhanced ocean survival.

Because available data bearing on this point (Table 2) were not originally collected with this analysis in mind they have some obvious deficiencies, yet they show some interesting features. The smolt samples from the Egegik system are too scant to be relied upon. For the other systems the largest smolts are from the Naknek River which includes smolts from both Brooks and the other lakes of the Naknek system. Comparison of Naknek-Brooks Lakes with Wood and Ugashik Lakes (Table 2) shows that smolt length appears to be definitely related to fertility. The possible long-term effects of volcanic enrichment are also indicated by the concentrations of chemicals after 35 years (1912–1947) in Brooks and Naknek Lakes, the only two in Bristol Bay benefitting from the Katmai outfall. In addition to the leaching of the soil in the usual manner, Naknek Lake still receives via the Savonoski River great torrents of pumice from the northern slopes of Mount Katmai. The chemical composition of this great volume of volcanic debris is a point

TABLE 2. *Limnological data and length of seaward-migrating sockeye smolts in Bristol Bay*[1]

River system	Sockeye seaward migrants in second year		Limnological data					
					Chemical analyses, ppm			
	Number	Length mm	Lakes sampled	Plankton volumes[2] cc/m³	Total hardness, as $CaCO_3$	Fixed carbon dioxide	Silica	Nitrite nitrogen $\times 10^{-3}$
Kvichak			Iliama	—	18.8	15.0	1.2	1.0
Egegik	36	96.4	Becharof	2.0	26.6	22.4	0.2	1.0
Naknek	1837	101.9	Naknek	—	77.7	36.4	8.1	0.2
Do.			Brooks	20.0	37.2	32.7	9.1	6.0
Naknek summary		101.9		20.0	37–38	33–36	8–9	0.2–6.0
Ugashik	221	58.0	Ugashik	4.0	21.1	22.0	4.0	0.2
Wood	3074	60.1	Wood	12.5	20.3	11.2	3.2	1.0
Wood-Ugashik summary		58.0–60.1		4.0–12.5	20–21	11–22	3–4	0.2–1.0

[1] Smolt lengths in 1939, limnological data (surface) in 1947.

[2] Horizontal tows with standard Birge net.

deserving investigation, especially since lake fertilization is under study as a possible conservation measure.

The data available, although not sufficient to prove the beneficial effects of volcanic activity on the salmon runs, are highly suggestive. Study of the age, chemical composition, distribution, and thickness of ash layers in this area of Alaska, might aid in solving some of the questions concerning long-term trends in abundance of salmon.

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Biological Factors in Treatment of Raw Sewage in Artificial Ponds

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ABSTRACT

Ninety-nine communities in the northern plains area use artificial ponds as the sole method of sewage treatment. Five ponds, selected for variability in design and loading, were studied intensively during each season for one year.

A typical biota principally composed of algae and bacteria becomes established and participates in the treatment process. Reduction in coliform density (most probable number) was 99% more than half the time and always 95% or greater. Oxygen demand (B.O.D.) was typically reduced by 70–90%. Total suspended matter was reduced 80% or more in winter, but sometimes was increased up to 258% in summer as result of algal production.

Various factors affect the treatment process. Depth of the euphotic zone ranged from 0.1–0.7 meter. Bottle tests gave oxygen production rates between zero and 3.6 g/m²/hr at different times of day, and total respiration rates from 0.1–2.4 g/m²/hr. Oxygen demand from contributed sewage was only 6–7% of total respiratory demand. Vertical gradients, diurnal variations, ice cover, and other factors prevent simple projection of bottle test data to the ponds.

INTRODUCTION

In the northern plains area draining to the Missouri River 99 communities use artificial ponds as the sole method of sewage treatment. These communities, which represent a total population of about 125,000 persons, are distributed by States as follows:

Colorado	9	Nebraska	3
Kansas	1	North Dakota	38
Minnesota	2	South Dakota	19
Missouri	7	Wyoming	5
Montana	15		
		Total	99

Depending upon community population these ponds range in size from 1/7 acre to 130 acres. Because of their low cost they have become extremely attractive to the small municipality in which per capita cost of conventional treatment is considerably greater than in larger municipalities. New sewage ponds can be built for about $4.50 to $20.00 per capita, or about 1/8 to 1/2 the cost of a conventional plant. In keeping with present design practice, artificial ponds provide one acre four feet deep for each 100 persons of connected population.

Reduced to simplest terms the stabilization mechanism in a sewage pond is mutually beneficial interaction between bacteria, algae, and various other plankters, which eliminates the objectionable characteristics of sewage. Digestive and oxidative activities of bacteria stabilize organic matter and simultaneously produce CO_2, ammonia, and other nutrients needed by the algae. The latter, through photosynthesis, liberate excess oxygen which helps to maintain a desirable aerobic system. In the overall process relatively unstable organic matter is converted principally to a more stable algal mass. These interrelationships between algae and bacteria have been studied intensively in California by means of pilot plant facilities that have a much shorter detention period than ponds used in the northern plains area (Ludwig and Oswald 1952, Oswald and Gotaas 1955).

Growth in popularity of raw sewage ponds in the northern plains area since 1950, and evident interest in using them shown by municipalities in other regions, emphasize the urgent need for better information on

[1] At time of the survey reported here, M. O. Allum was on staff of the South Dakota State Health Department.

how they function, how they should be constructed, and how their efficiency might be improved. Accordingly, a seasonal study of five ponds was made during the period January 1955 to June 1956 as a cooperative undertaking of the Public Health Service and the State Health Departments of North and South Dakota. This field survey is the initial phase of a continuing comprehensive study of pond treatment of wastes. The objectives were to evaluate structural, seasonal, and loading variations and to point up areas of approach and direction to be taken in pilot plant studies. The material presented in this paper is based entirely upon observations made during the initial field study.

Of five raw sewage ponds studied during each season of 1955, two have been selected for presentation because they represent the lowest and the highest B.O.D. loadings encountered. The pond at Lemmon, South Dakota, with an area of 25 acres serves 2,600 persons at a mean B.O.D. loading of 6.84 pounds/acre/day. At Kakoka, South Dakota, the raw sewage pond has an area of 3.0 acres and serves 800 persons at a mean B.O.D. loading of 22.9 pounds/acre/day.

Various factors, such as loading, solar radiation, depth, wind, temperature, and ice cover, influence the course and velocity of the stabilization process. Although these factors have profound effects upon various physiological reactions, and some of these are partially known, impact upon photosynthesis is of greatest immediate interest and importance.

DATA

The results of pond treatment are impressive. B.O.D. concentrations typically are reduced in a seasonal range of 70–98%. Reductions in coliform bacterial densities, in terms of M.P.N., can be expected to exceed 95% at all times and 99% more than half the time. Total suspended matter was reduced 80% or more in winter, but sometimes was increased up to 258% in summer as a result of algal production. Freedom from odor nuisances, scarcity of floating solids, and the green natural appearance do not suggest a sewage origin of the pond content.

Continuing emphasis is placed upon photosynthesis in aquatic communities in relation to primary production of organic matter (Steeman-Nielsen 1937, Ryther 1954, 1956a, 1956b, Verduin 1951, 1956). As a rule in such studies oxygen production is mainly significant as a step toward the measurement of primary production. In contrast to this, photosynthetic oxygen evolved in raw sewage ponds is a more important consideration than the organic matter produced concurrently in the process. Effectiveness of oxygen production has a practical value in relation to sewage loading and optimum depth. Although oxygen production in relation to light and depth is considered here solely from this point of view, considerable attention has been directed by others to cultivation and harvesting of algae grown in sewage (Gotaas, Oswald and Ludwig 1954).

Three distinct considerations are involved in the importance of solar radiation and help determine pond efficiency and the area and depth needed. First, regional variations in annual solar radiation, which are governed by latitude, elevation, and cloud cover, will influence pond design in a given location. Second, seasonal changes in daily solar radiation, which may vary locally by a factor of three or more, suggest the seasonal difficulties to be expected. Finally, penetration of incident light determines how much of the pond volume will participate in oxygen production.

Penetration of light in raw sewage ponds is strikingly less than in most bodies of water for which data are available (Clarke 1939, Birge and Juday 1929–32, Whitney 1938). Figure 1 shows light penetration in three South Dakota bodies of water, as measured by matched surface and submerged photronic cells (Weston, Model 856 RR) sensitive in a range from 420–700 mμ. The transparency of Murdo Lake, which is a water supply reservoir, is not unusual with an extinction coefficient of 0.58 per meter. In this lake, the rate of light absorption increased with depth. The euphotic zone,

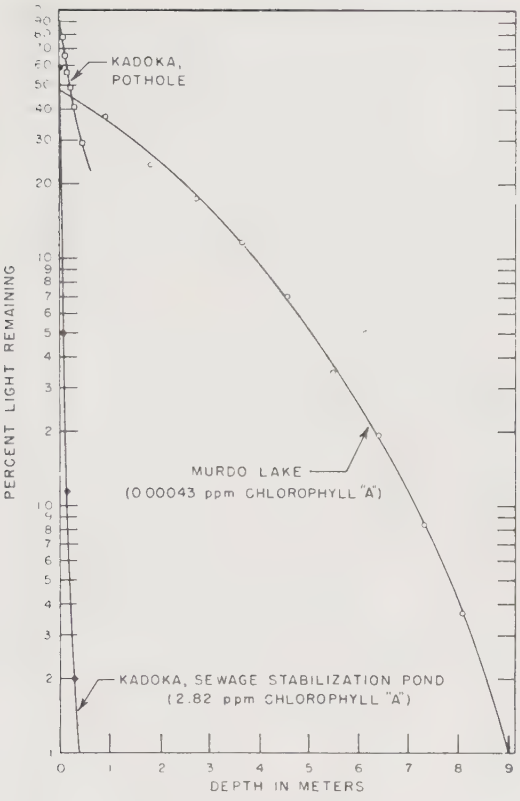

FIG. 1. Light remaining at various depths in three different bodies of water, July 27–29, 1955.

the lower boundary of which by definition is located at 1 % of surface light, extended to a depth of 7.16 meters. In the sewage stabilization pond at Kadoka the corresponding depth was only about 0.4 meter! There is little doubt that dense algal populations in this and other highly fertile raw sewage ponds are responsible for the rapid light extinction observed. Systematics and growth features of algae in somewhat similar sewage oxidation ponds in California have been studied intensely by Silva and Papenfuss (1953) and Allen (1955).

Because of these interesting light relations and the current practice of constructing raw sewage ponds with an average depth of 4 feet (1.22 meters), it seemed desirable to estimate photosynthetic oxygen production as a step in evaluating this benefit to treatment from each increment of excavated depth. A sample of sewage pond content

TABLE 1. *Oxygen production at different depths 10:00 AM–3:00 PM, August 12, 1955, at Lemmon, South Dakota*

Determinations were made in triplicate

% surface light	Approx. ft. candles	Depth cm	D.O. change, ppm		Oxygen production ppm/hr	Respiration ppm/hr
			Light bottles	Dark bottles		
19.0	1050	25.4	12.6	—2.1	2.9	0.4
3.1	167	60.9	0.0	—2.1	0.4	0.4
0.5	27	96.5	—1.4	—2.0	0.1	0.4
0.07	3.8	134.6	—1.6	—1.8	0.04	0.4

taken from a depth of 20 cm was distributed into 2 series of 300-ml glass-stoppered bottles, which were suspended at selected depths in chicken-wire baskets. Half the bottles at each depth (usually duplicates or triplicates) were covered with two layers of aluminum foil to exclude light. At termination of the exposure period dissolved oxygen concentrations were determined using the Alsterberg (Azide) modification of the Winkler test (APHA, AWWA, and FSIWA 1955) for comparison with initial values similarly obtained.

In one test of five-hours' duration at the Lemmon Pond (Table 1), bottles were hung at four depths giving a range in mean light intensity from 3.8 to 1050 foot-candles. Oxygen production at 25.4 cm was seven times as great as at 60.9 cm, and 29 times as great as at 96.5 cm. At 83.0 cm, at which 1 % of surface light remained, oxygen production was estimated to be only 0.2 ppm/hr. If the production rate between 25.4 cm and the surface is taken arbitrarily as 2.9 ppm/hr, the production in the euphotic zone was then 1.2 $g/m^2/hr$ (or 9.2 pounds/acre/hr), based upon a mean production of 1.4 ppm. Simultaneous respiratory demand in the euphotic zone was 0.3 $g/m^2/hr$, or only 25 % of the total production. This relationship is not actually as favorable as it appears, as will be shown in subsequent discussion.

Verduin (1956) has found that the ratio of mean photosynthesis in the euphotic zone to the rate under optimal light conditions is 0.65. If that ratio applies to the present test conditions, and if 2.9 ppm/hr is

assumed to approximate the rate under optimal light, the resulting mean production rate in the euphotic zone would be 1.9 ppm/hr. This compares favorably with the estimated weighted average of 1.4 ppm/hr determined from measurements at several depths. Also, using Verduin's listed yield of 0.22 micromoles of oxygen evolved per microgram of chlorophyll per hour, the calculated sewage pond yield under optimal light, with 0.46 ppm of chlorophyll, would be 3.2 ppm/hr. These, of course, are optimal rates of production which do not prevail throughout the day as shown in tables that follow.

Raw sewage ponds differ from other standing bodies of water in the intensity of photosynthesis and respiration in the shallow surface layer and the rapidity with which light decreases with depth. At no time was a pond found with a euphotic zone deeper than 0.7 meter, and one as shallow as 0.1 meter was measured. Difficulties in suspending light and dark bottles at precise light levels and in determining exact depth of optimal light are apparent.

Tests similar to that described above were done during four different daylight periods in ponds at Lemmon and at Kadoka. At Lemmon (Table 2), where the euphotic zone was then about 83 cm thick, bottles were hung at 25.4, 60.9, and 96.5 cm to give 19.0, 3.1, and 0.5% of incident light intensity, respectively. A separate pond sample was used for each test. Variations resulting from this procedure are suggested by differences in the respiratory values.

TABLE 2. *Relation of photosynthesis to respiration at different times of day, August 10, 1955, at Lemmon, South Dakota*

Time of day	Duration of measurement, hours	Light intensity ft. cand.		Dissolved oxygen relations gm/m²/hr			
		25.4 cm	9.65 cm	Total photosynthetic production	Total respiratory use	Surplus	Observed sewage influent B.O.D. loading (ultimate)
9:00 AM	0.5	552	5.5	2.1	0.9	1.2	0.04
1:00 PM	1.0	780	7.8	1.5	0.7	0.8	0.04
3:00 PM	2.0	390	3.9	0.8	0.7	0.1	0.04
7:30 PM	2.0	55	<1.5	0.03	0.6	−0.6	0.04

Oxygen production varied from 2.1 g/m²/hr at midmorning to 0.03 g/m²/hr during early evening. Cause for the lower production at 1:00 P.M. than at 9:00 A.M. is not definitely known but very likely resulted from depletion of available CO_2. However, if total respiratory use largely reflected phytoplankton density at the depth of sampling, oxygen production per algal quantity during the two periods was not greatly different.

To evaluate the photosynthetic system in relation to the sewage load for which oxygen must be furnished, daily ultimate B.O.D. load was simply converted to grams of oxygen demand/m²/hr. While admittedly this is not a precise procedure because sewage flow fluctuates widely and the oxygen demand of the sewage is not identifiable in the complex demand of the pond system, it is sufficiently accurate to permit gross comparison of oxygen demand from sewage with that of total respiration. At first glance it seems highly inefficient to create a biological system with an oxygen use rate of 0.7 g/m²/hr in order to obtain the 0.04 g/m²/hr needed to stabilize the sewage. This relationship is shown graphically in Figure 2. Cheap land, low construction costs, and limited maintenance requirements make this apparent inefficiency of less consequence than would otherwise be the case.

Similar bottle tests were conducted in the raw sewage pond at Kadoka. As shown in Table 3 sewage loading was twice that

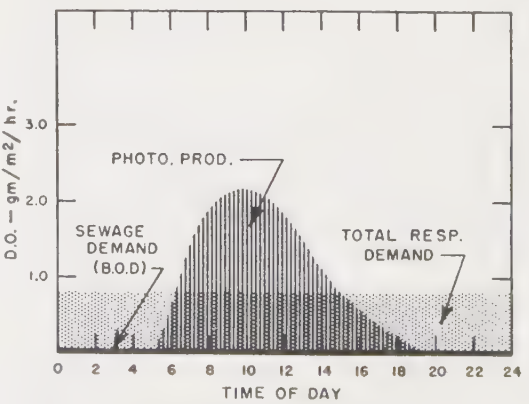

FIG. 2. Oxygen relations in a typical raw sewage pond.

TABLE 3. *Relation of photosynthesis to respiration at different times of day, July 28, 1955, at Kadoka, South Dakota*

Time of day	Duration of measurement, hours	Light intensity ft. cand. 7.6 cm	Light intensity ft. cand. 43.2 cm	Dissolved oxygen relations gm/m²/hr Total photosynthetic production	Total respiratory use	Surplus	Observed sewage influent B.O.D. Loading (ultimate)
9:30 AM	0.5	945	2.7	3.6	1.9	1.7	0.09
2:00 PM	0.5	2830	8.1	2.3	2.4	~0	0.09
4:30 PM	0.5	750	2.1	2.4	1.2	1.2	0.09
8:30 PM	0.5	<1.5	<1.5	0.0	0.1	~0	0.09

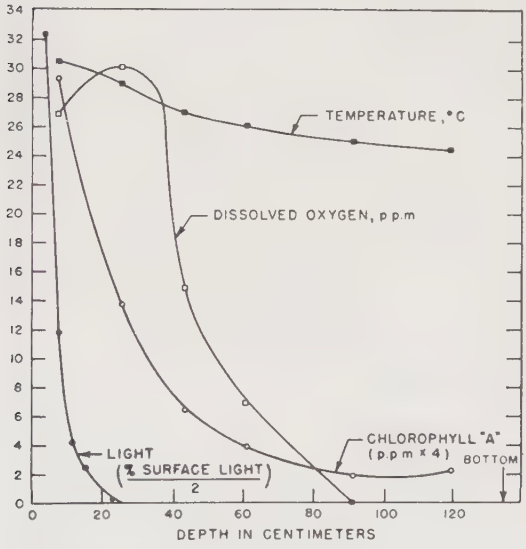

FIG. 3. Vertical variations in water quality characteristics, 12:15–12:30 P.M. July 28, 1955, Kadoka, South Dakota. Values of the ordinate apply to temperature and dissolved oxygen directly and to chlorophyll "a" and light as indicated.

TABLE 4. *Chlorophyll "a" concentrations (in ppm) at various depths at different times of day, July 27, 1955, Kadoka, South Dakota*

Depth in cm	10:45 AM	12:30 PM	5:45 PM
7.6	—	7.32	3.28
25.4	—	3.45	—
30.5	3.36	—	—
43.2	—	1.62	2.62
60.9	2.86	0.99	1.53
91.4	2.22	0.49	0.92
119.4	—	0.38	0.53

TABLE 5. *Chlorophyll "a" Concentrations (in ppm) July 29–August 20, 1955*

	7.6 cm depth	121.9 cm depth
Kadoka, South Dakota	2.82	0.35
Wall, South Dakota	0.18	—
Lemmon, South Dakota	0.47	0.44
Maddock, North Dakota	0.08	0.06
Wishek, North Dakota	0.65	1.05
Murdo Lake, South Dakota	0.0004	0.0002

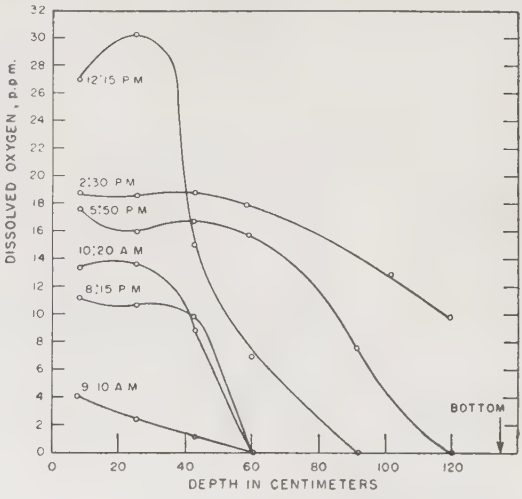

FIG. 4. Vertical variations in dissolved oxygen at different times of day, July 28, 1955, Kadoka, South Dakota.

observed at Lemmon, and both oxygen production and total respiration were more intense in response to the heavier loading.

Although bottle-test data have been treated as if they apply directly to the ponds, there are many environmental differences between them that must be reconciled. Some of these are related to a procession of diurnal changes. Pond temperature, for example, follows changes in air temperature in a cyclic fashion. Temperature gradients, as shown in Figure 3, generally are established on calm days and disappear readily during windy weather and gradually after sunset as do those classified in Pichler's "Small ponds" category (Young and Zimmerman 1956).

Vertical distribution of phytoplankton as indicated by chlorophyll concentration also follows a diurnal pattern in which concentration near the surface at mid-day may be strikingly greater than a few centimeters

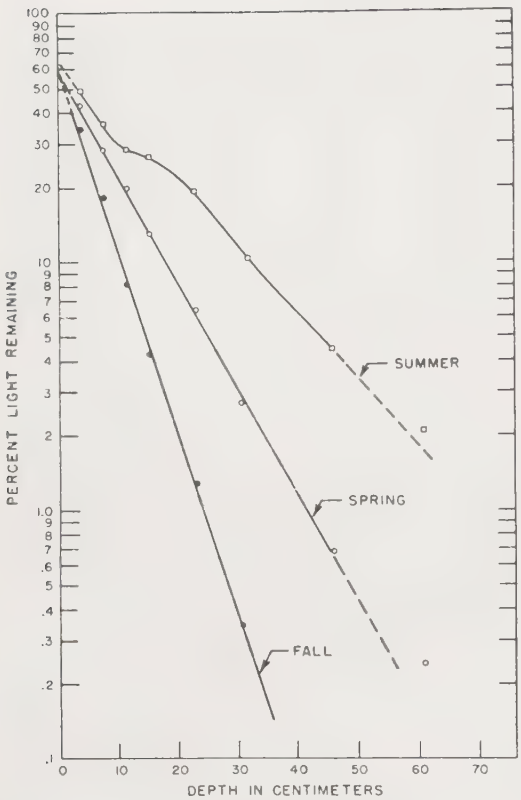

FIG. 5. Light remaining at various depths during spring, summer, and fall, 1955, Lemmon, South Dakota.

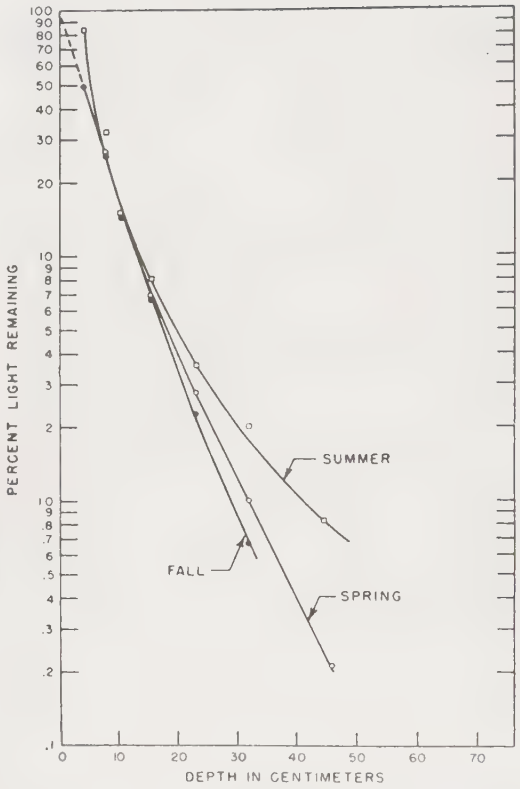

FIG. 6. Light remaining at various depths during spring, summer, and fall, 1955, Kadoka, South Dakota.

deeper. This pattern is suggested by the data in Table 4, and is further supported by chlorophyll determinations on samples of pond overflow collected at three-hour intervals over a 24-hour period. Table 5 gives the range of chlorophyll concentrations found in the bodies of water sampled. As one would expect, chlorophyll concentration increases with increased loading.

Dissolved oxygen concentrations are more transient in the ponds than in the test bottles. During a 24-hour period dissolved oxygen concentrations at a given depth were observed to vary from zero to 35 ppm or more in following a daily repetition of mid-day highs and nocturnal lows. At one extreme oxygen bubbles escape from the pond surface, at the other anaerobic conditions prevail. At mid-day, vertical variations have been found to range from 30.3 ppm at 25.4 cm to zero at 91.4 cm. As shown in Figure 4, the vertical and diurnal patterns result from different rates of gain and loss at each depth. Because the euphotic zone extended to only 40.6 cm at this time, oxygen gain below 60 cm after mid-day was most probably the result of diffusion from the highly productive overlying layer.

Mainly because of seasonal changes in phytoplankton density, light absorption and thickness of the euphotic zone change with season and even from day to day. Light absorption characteristics during spring, summer, and fall are shown for the Lemmon pond in Figure 5, and for the Kadoka pond in Figure 6. Deviations from a straight-line plot are caused by uneven vertical distribution of phytoplankton as described. Limits of the euphotic zone at Lemmon were more variable than at Kadoka.

In all ponds studied transparency was less during spring and fall than during summer. This is shown in Table 6, which also gives

TABLE 6. *Approximate depth in cm at which indicated light remains*

	10% of Surface Light	1% of Surface Light	Extinction Coefficient*
Kadoka, South Dakota			
Spring	12.7	31.8	14.8
Summer	14.0	40.6	9.6
Fall	12.7	29.2	16.0
Wall, South Dakota			
Spring	5.1	10.2	46.0
Summer	17.8	41.9	10.7
Fall	5.1	15.2	22.9
Lemmon, South Dakota			
Spring	17.8	41.9	9.9
Summer	33.0	69.9	5.9
Fall	10.2	24.1	17.4
Maddock, North Dakota			
Spring	14.0	30.5	13.7
Summer	25.4	58.4	7.0
Fall	19.1	39.4	10.8
Wishek, North Dakota			
Spring	7.6	17.8	22.4
Summer	16.5	39.4	10.8
Fall	8.9	21.6	18.4
Kadoka, South Dakota, Pothole			
Summer	—	—	2.55
Murdo Lake, South Dakota			
Summer	4,013	7,162	0.58

* Extinction coefficient = k in equation $I/I_0 = e^{-kl}$ with l expressed in meters.

(Table 7). These figures agree closely with measurements by Greenbank (1945) on several southeastern Michigan lakes. He noted that "cloudiness" of the ice caused by minute air bubbles or dirt had great influence upon light transmission. The contrast in per cent transmission between Lemmon and Kadoka similarly is the result of clear ice at the former and opalescent ice at the latter. In these observations ice quality appeared more important than thickness, and quality generally increased as B.O.D. loading decreased.

SUMMARY

Dissolved oxygen relations are the key to freedom from nuisance conditions and acceptibility of raw sewage ponds as municipal treatment facilities. Light and dark bottle tests offer one means of exploring oxygen relations as affected by controllable factors such as loading and depth. Estimated oxygen production, using the factors given by Verduin (1956), agrees fairly well with the test data. Ryther's (1956a) expression for determining absolute photosynthesis in the ocean, and pond design equations that have been developed (Gotaas, Oswald, and Golueke 1954) are other approaches to be explored in raw sewage ponds. Vertical variations in pond content, diurnal and seasonal effects, weather changes and other factors, emphasize the restraint with which such data may presently be applied.

TABLE 7. *Light transmission through ice and snow, January 22–February 10, 1955*

Sewage stabilization pond	Date	Mean ice thickness cm	Mean snow thickness cm	Mean surface light ft. cand.	Mean light transmission per cent
Kadoka, South Dakota	Jan. 22	26.7	2.3	1086	6.5
Wall, South Dakota	Jan. 25	25.6	0.0	608	20.4
Lemmon, South Dakota	Jan. 31	32.3	0.0	1337	55.5
Maddock, North Dakota*					
Cell 1	Feb. 5	66.0	7.6	1300	1.2
Cell 2	Feb. 5	66.0	7.6	1460	5.1
Cell 3	Feb. 5	62.2	5.1	1100	3.5
Wishek, North Dakota	Feb. 10	43.2	7.6	1490	1.2

* Installation consists of three ponds or cells connected in series.

the limits of the euphotic zone and the extinction coefficient for each body of water.

There is generally no measurable oxygen production during winter under ice, and consequently anaerobic conditions are continuous from the onset of ice cover to ice breakup. It is not possible to discern clearly each causative factor from the others, but light extinction alone would drastically limit photosynthesis even if other factors were favorable.

Depending upon thickness and variation in clarity, light-transmission through ice and snow ranged from 55.5% to 1.2%

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Primary Production Measurements in Eleven Florida Springs and a Marine Turtle-Grass Community[1]

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ABSTRACT

During July and August, 1955, primary production measurements were made in eleven Florida spring communities and a marine turtle-grass community in the Florida Keys by means of the diurnal curve method. Diurnal measurements of oxygen and carbon dioxide made at a station downstream from the main springs were used in estimating gross primary production and community photosynthetic quotients. These curves show in detail the course of production hour by hour under various conditions in whole natural communities.

The primary production values obtained ranged from 0.7 g oxygen/m²/day in a small, heavily shaded, anaerobic spring on a rainy day to 64 g oxygen/m²/day on a sunny day in an aerobic spring where the plant beds trailed at the water surface. A comparison of the chemostatic properties in the springs studied suggests that oxygen, phosphate, nitrate, and carbon-dioxide levels are relatively unimportant in determining the magnitude of primary production in these spring communities. Light as influenced by cloud cover, trees, and water depth is the main controlling factor. Approximate efficiencies found were 0.5 to 10% (mean 4%) of the visible light energy reaching plant level. Downstream increases in planktonic chlorophyll and oxygen suggested a steady state in 7 km of longitudinal succession in Rainbow Springs River. Net production of benthic algae of 1.5 g/m²/day was estimated in Orange Springs from the rate of bubble release into funnels placed on the bottom.

INTRODUCTION

The large artesian springs of Florida are collectively a giant laboratory for the study of natural communities. All are maintained at constant temperature and relatively constant chemical condition by the strong flow from underground. Although the temperatures of about 23°C are similar, the chemical conditions of each spring are somewhat different (see Ferguson *et al.* 1947). Since many properties are similar from spring to spring, it is possible to compare the productivity of springs in an effort to determine the effects of those factors that do differ.

In a previous paper (Odum 1956a) some methods were described for measuring primary production in flowing waters from an analysis of diurnal curves for oxygen and carbon dioxide. The results of testing this method closely in Silver Springs, Florida, are reported elsewhere (Odum 1957). In this paper similar studies are reported for eleven other Florida Springs, as well as a marine turtle-grass community whose tidal waters flow over the benthic community in a way that suggests comparison with the freshwater spring communities. Some properties of these thermostatic and chemostatic communities are given in Table 1.

The diurnal curve method of measuring production as applied to springs consists of measuring oxygen and carbon dioxide throughout a day at a station below the spring outflow (boil). Since the boil is constant in properties, the action of the community on the water is indicated by the downstream differences. By subtracting the night values from the day values and applying a correction for diffusion differences between day and night, one obtains the gross primary production.

In some streams the changing degree of saturation at night permits an estimate of diffusion and respiration to be obtained from the diurnal curves themselves (Odum

[1] These studies carried out at the University of Florida were aided by a contract NR 163-106 between the Biology Branch, Office of Naval Research and the Department of Biology, University of Florida. The author is grateful to James B. Messerly for his effective research assistance in carrying out the field collections and analyses.

TABLE 1. *Characteristics of the communities studied*[a]

Name of spring and location	Depth of plants[b] m	Shading by trees[b] %	Oxygen[a] ppm	Carbon dioxide ppm	NO_3-N ppm	PO_4-P ppm	N/P by atoms	Area of measurement zone[c] $\times 10^3 m^2$
Anaerobic Springs								
Beecher Springs, Welaka, Putnam Co.	1	80	0.8	4.5	0.11	0.112	2.3	2.9
Blue Springs, Volusia Co.	1	50	0.25	7.9	—	0.123	—	16
Aerobic Springs								
Weekiwachee Springs, Hernando Co.	2	10	1.3	4.8	0.12	0.018	15	16
Manatee Springs, Levy Co.	0.5	60	1.8	13.2	—	0.037	—	6.6
Silver Springs, Marion Co.	1	10	2.6	9.7	0.46	0.054	23	76
Green Cove Springs, Clay Co., zone below pool	0.05	50	2.8	1.8	0.09	0.005	42	0.43
Chassahowitzka Springs, Citrus Co., oligohaline side boil	0.1	10	3.8	9.2	—	0.013	—	4.0
Blue Spring, Alachua Co., small side boil with *"Utricularia"*	1	90	3.9	7.5	—	—	—	1.4
Blue Spring, Alachua Co., main boil	0.2	70	4.2	6.8	0.85	0.092	21	2.6
Homosassa Springs, Citrus Co., fish-bowl spring, oligohaline	0.1	30	4.3	4.9	0.080	0.008	23	3.6
Rainbow Springs, Marion Co.	1.5	10	5.2	4.9	0.080	0.008	23	3.6
Marine Turtle Grass,								
Long Key, Fla.	0.5	0	4.2	—	—	—	—	—

[a] The springs are arranged in order of increasing oxygen concentration of the water (nighttime values).

[b] Visual estimation.

[c] From boil to downstream measurement station.

1956a). However, in springs with their constant outflows, diffusion must be determined independently. In this paper a value of the diffusion correction obtained in work in Silver Springs (Odum 1957) is used. The diffusion estimate itself, however, is not crucial for the determination of gross primary production. Diffusion is subtracted along with respiration when the night values are subtracted from the daytime values. It is only the change in diffusion resulting from the change in saturation deficit for which correction must be made. Judging from studies on Silver Springs, the correction for changing diffusion apparently amounts to only about 10 to 15% of the total production in spring communities.

In this paper as in previous papers small letters designate metabolic rates on a water-volume basis and capital letters metabolic rates on a community-area basis.

CHEMICAL METHODS

Oxygen was measured by the unmodified Winkler method, the samples being fixed in the field. Carbon dioxide was determined from pH and methyl purple alkalinity by means of an alkalinity nomogram (Hirsch 1945: 288). Because of the low organic matter content of the water (less than 1 ppm) it was possible to bring the water back to the laboratory for pH determinations. These were made within 12 to 24 hours of the time of sampling. Chlorophyll determinations of the plankton were made from 3-liter samples. After the water was passed through millipore filters, the filter was then dissolved in acetone and the pigment concentration estimated with a Beckman Spectrophotometer following the procedure of Richards with Thompson (1952).

THE DIURNAL CURVES OF OXYGEN AND CARBON DIOXIDE

In Figures 1–10 are given the diurnal curves made in 10 springs at stations located

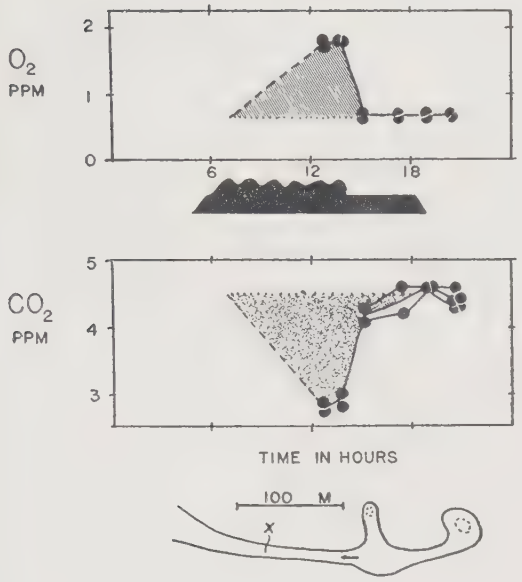

FIG. 1. Diurnal curves of oxygen and carbon dioxide at a downstream station marked *x* in Beecher Springs, Welaka, Putnam County, Florida, August 2, 1955. This spring is anaerobic and characterized by blue-green algae and white sulfur bacteria.

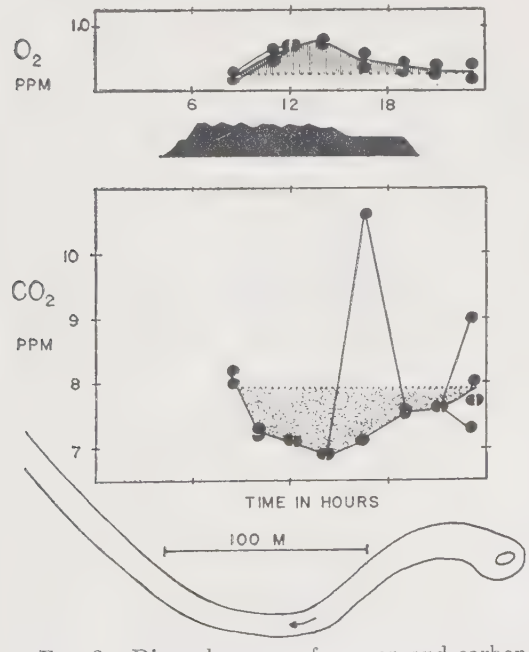

FIG. 2. Diurnal curves of oxygen and carbon dioxide from a station 450 meters downstream in Blue Springs, Volusia County, Florida, August 9, 1955. The spring is anaerobic and characterized by blue-green algae.

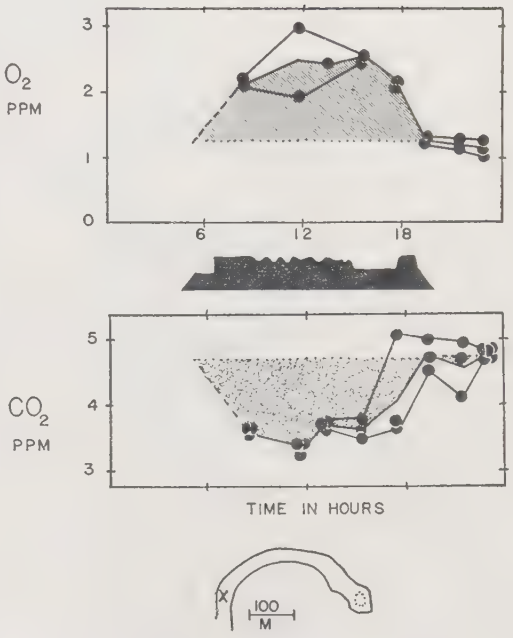

FIG. 3. Diurnal curves of oxygen and carbon dioxide at a station marked *x* downstream from Weekiwachee Springs, Hernando County, Florida, July 26, 1955. The spring is very deep and floored with *Sagittaria* and algae, especially *Hydrodictyon*.

in each case a short distance below the main boil. For reasons of economy complete 24-hour series were not obtained, but the field work was planned so that a representative night value was obtained either late at night or early in the morning. All sampling was done in duplicate or triplicate. The shaded areas between the mean curves and the horizontal lines representing the night values are a measure of the gross production. The lower part of the horizontal black bar graph indicates the duration of light. The upper margin of the black bar is straight and horizontal when the sun was shining directly without interference from clouds as indicated by descriptive notes made in the field. It should be realized that the curves are records of the hour to hour changes in production, which reflect the weather and cloud changes intimately. No clear cut evidence was found of a midday depression in production as known for isolated land plants.

As also reported in the Silver Springs

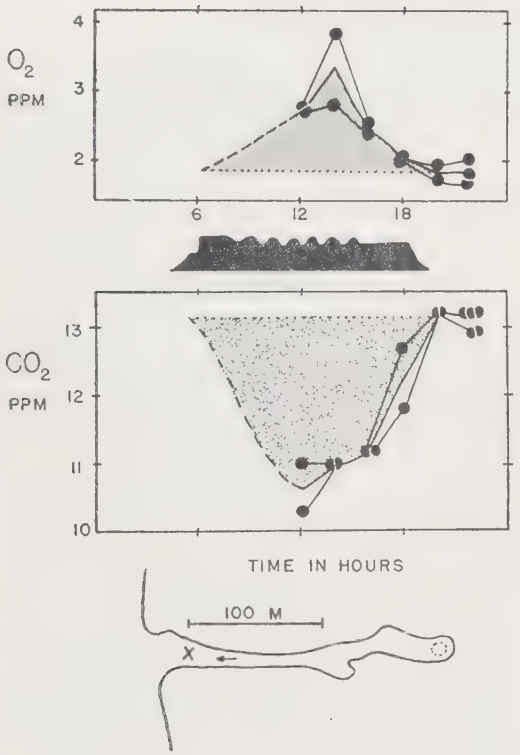

FIG. 4. Diurnal curves of oxygen and carbon dioxide at a downstream station marked x in Manatee Springs, Levy County, Florida, August 15, 1955.

work, the oxygen and carbon-dioxide curves are not entirely symmetrical in that the carbon-dioxide curves show irregular behavior after sunset. Whereas the oxygen release by the community ceases at sunset, the carbon dioxide uptake seems to continue longer as indicated in the graphs for Rainbow, Homosassa, Beecher, and Blue Springs (Figs. 1, 2, 9, and 10). In contrast, the Alachua Blue Springs (Fig. 7) show some kind of over-compensation after sunset with the carbon dioxide rising temporarily to a higher value than typical of the nighttime. It is not certain whether these effects represent the behavior of the plants or are an artifact due to some differences in mixing that occur along the shores as some of the backwaters cool.

In Green Cove Springs (Fig. 5) the water from the boil flows in part into a swimming pool and over a falls before flowing over the community of organisms in the spring stream. In this situation the large volume of the swimming pool relative to the flow introduces complex lag factors affecting the water that enters the downstream zone. In such a situation where the properties of the water entering the zone are not constant the two-station analysis must be resorted to. As shown in Figure 5, diurnal curves were obtained at both ends of a short segment of the stream. The upstream values were then subtracted from the downstream values to get the rate-of-change curves for oxygen and carbon dioxide.

In Figure 11 are presented data for marine turtle grass off Long Key, Florida, obtained by the modification of the diurnal curve method referred to previously as the spot method (Odum 1956a). The thick and characteristic beds of *Thallassia* were located in 3 ft of flowing water which was continually changing in direction and velocity because of the tide. The procedure used here was for the wading observer to follow a spot of fluorescein dye in the water for about ten to twenty minutes, using a flashlight at night. The enormously high production rate of this dense grass community produced significant oxygen changes in short time intervals. Duplicate samples of water were taken adjacent to the dye spot at the start and completion of a run. The fluorescein spot was renewed periodically from a dropping bottle as the dye dispersed. The oxygen values obtained are given in Table 2. An oxygen rate-of-change curve (Q) was computed as given in Figure 11. Respiration and diffusion were then obtained using the methods given previously (Odum 1956a). When these estimates of rates of respiration and diffusion are subtracted from the oxygen rate-of-change curve the primary production curve obtained is shown at the bottom of Figure 11. Because different degrees of saturation were found at different times of night, the change in oxygen rate-of-change had to be related to the change in saturation deficit in order to compute the diffusion coefficient. This was not possible in the case of the springs.

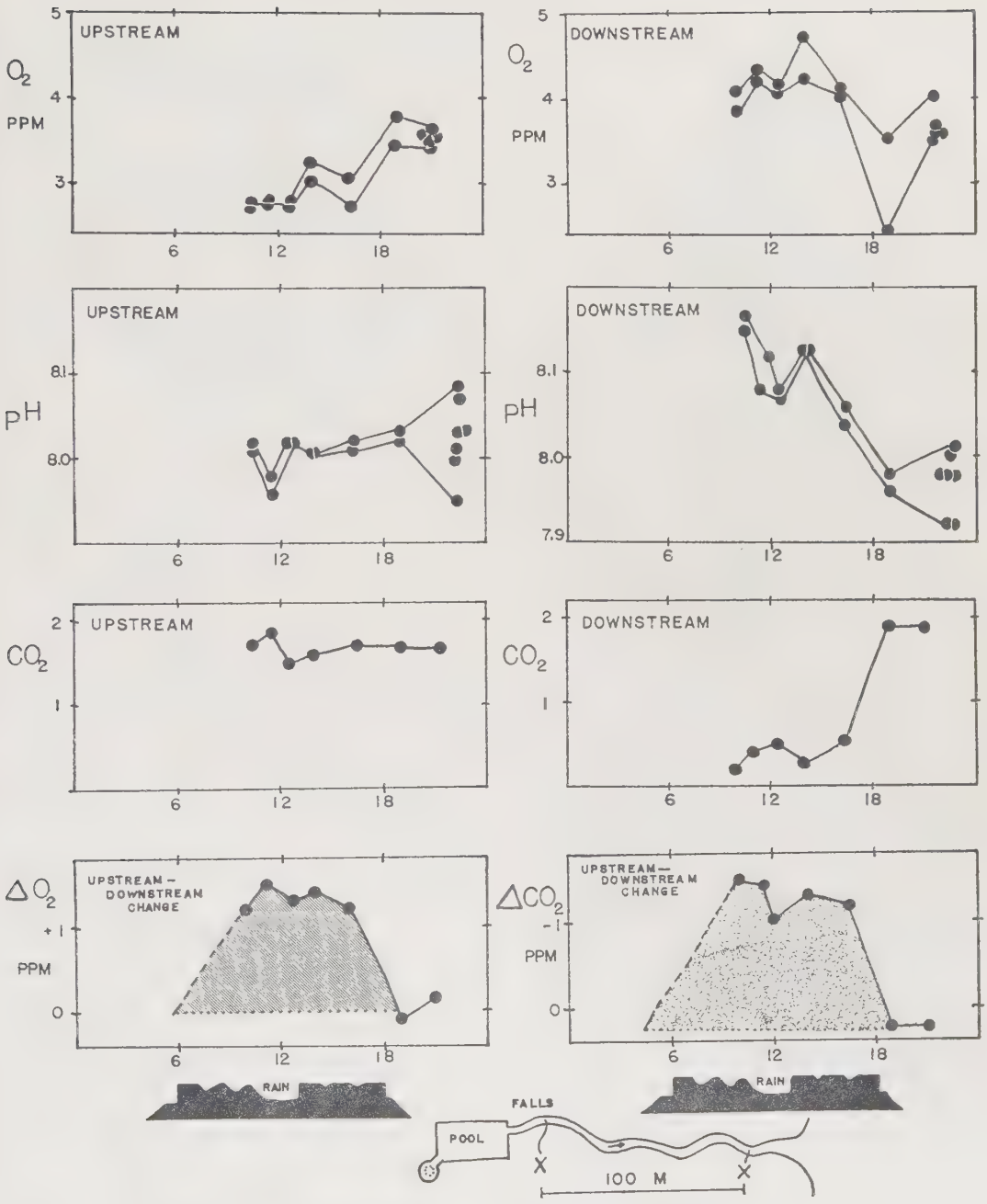

FIG. 5. Diurnal curves of oxygen, pH, and carbon dioxide as computed from pH at two stations (marked *x*) in the run below Green Cove Springs, Clay County, Florida, July 7, 1955. In the lower graphs are plotted curves of oxygen and carbon dioxide change between the upstream and downstream stations. This shallow brook contains *Vallisneria* beds.

COMPUTATION OF PRIMARY PRODUCTION AND COMMUNITY PHOTOSYNTHETIC QUOTIENTS

In Table 3 is listed the primary production computed for each pair of diurnal oxygen and carbon-dioxide curves. The computation consists in multiplying the area under the diurnal oxygen curve (Figs. 1–11) in ppm-hrs/day by the discharge in m^3/hr and

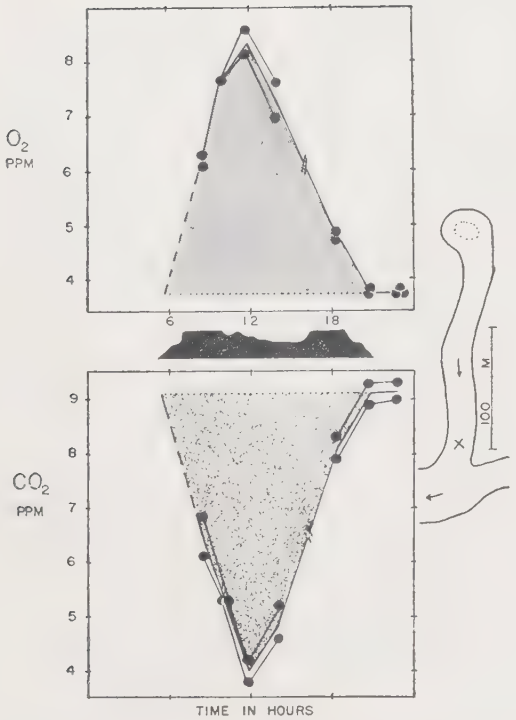

FIG. 6. Diurnal curves of oxygen and carbon dioxide at the mouth (marked x) of the first of the smaller oligohaline springs that flows into the main Chassahowitzka Springs run from the North a few hundred feet below the main fish-camp area, August 3–4, 1955. The water is filled with heavy beds of *Potamogeton pectinatus*, *Vallisneria*, and algae, which trail at the surface.

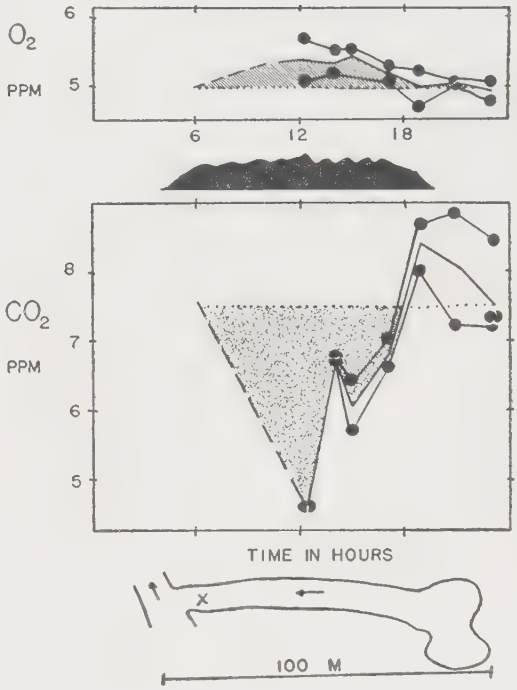

FIG. 7. Diurnal curves of oxygen and carbon dioxide at the mouth (marked x) of a small shaded boil full of *Utricularia* at Blue Springs, Alachua County, Florida, July 28, 1955.

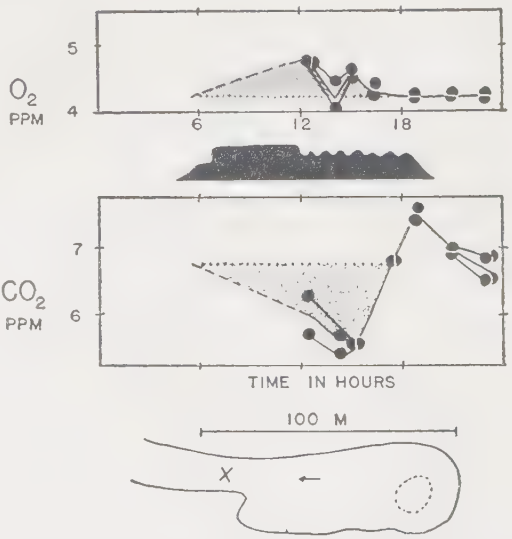

FIG. 8. Diurnal curves of oxygen and carbon dioxide at a station marked x in the main boil of Blue Springs, Alachua County, Florida, July 28, 1955. The spring contains mixed vegetation: *Vallisneria*, *Najas*, *Ceratophyllum*, and surface vegetation.

dividing by the area of the community between the boil and the downstream station in m². For example, in Weekiwachee Springs 14.5 ppm-hrs times 10.7×10^3 m³/day divided by 16×10^3 m² is 9.7 g/m²/day oxygen production. In the Silver Springs study the oxygen production was shown to be more closely proportional to the energy storage than was the dry weight or ash-free dry weight because of the possible shift from glucose to protein production with changes in light intensity. Consequently production values are reported as oxygen measured. Based on Silver Springs a 10% uniform correction for change in diffusion rate between night and day was added to obtain the production values in Table 3. Since the range in saturation deficit of oxygen during night and day is

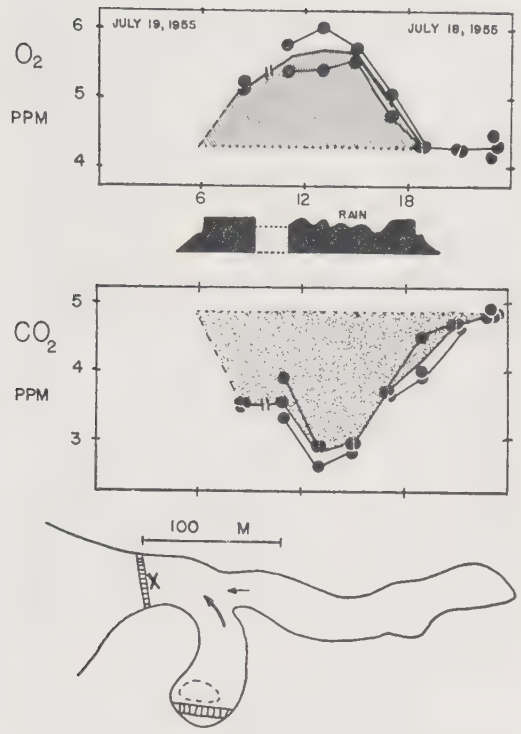

FIG. 9. Diurnal curves of oxygen and carbon dioxide at the station marked x in Homosassa Springs, Citrus County, Florida, July 19, 1955. Most of the discharge comes from the main fish-bowl boil as indicated by the heavy arrow in the sketch. The area of the small springs to the right is mostly shaded and covered with floating aquatics. Only the area of the main flow is used in computations. This main spring is oligohaline and perpetually a place of milling about for marine fishes.

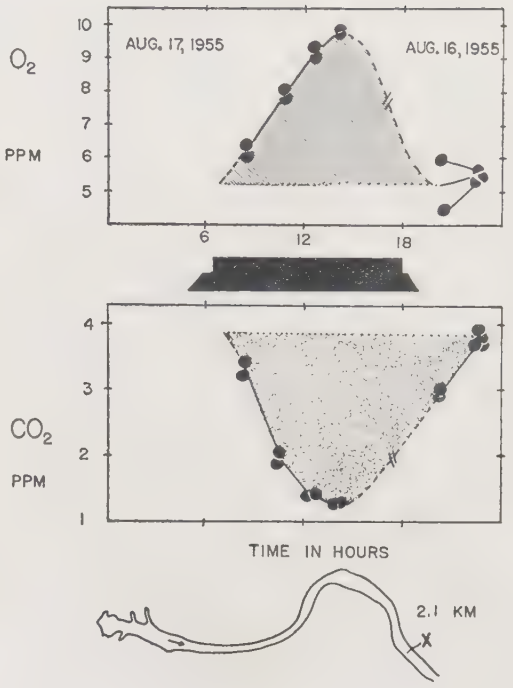

FIG. 10. Diurnal curves of oxygen and carbon dioxide at a downstream station marked x on a clear day in Rainbow Springs River, Marion County, Florida, August 16, 1955.

similar in each spring, the diffusion effects are likely to be similar.

The calculation of carbon-dioxide assimilation was made in the same way as described for oxygen. Then the ratio of the oxygen production to the carbon-dioxide production was reported as a community photosynthetic quotient as already done for Silver Springs (Odum 1957).

FACTORS AFFECTING PRIMARY PRODUCTION

In Table 1 are listed some of the main properties of the community that might be expected to modify the primary production: the degree of shading of the stream by trees, the approximate depth of the water to the main level of photosynthesis in the plant beds, the nitrate-nitrogen, the phosphate-phosphorus, the N/P ratio, the carbon-dioxide concentration, and the oxygen concentration.

It is clear from the diurnal curves and the computed production values in Table 3 that there is an enormous range of production within the springs studied. In considering the factors responsible for these differences, one should think of these communities as being in steady-state equilibrium due to a thermostatic, chemostatic, and remarkably constant biological environment. Thus the productions that are reported represent the productivity of the stabilized steady state and presumably represent the maximum production possible under the conditions of the particular outflow. Hypothetically communities will adjust to the maximum production that can be sustained since such a community may be expected to have maximum survival value following Lotka's maximum power principle (Odum and Pinkerton 1955).

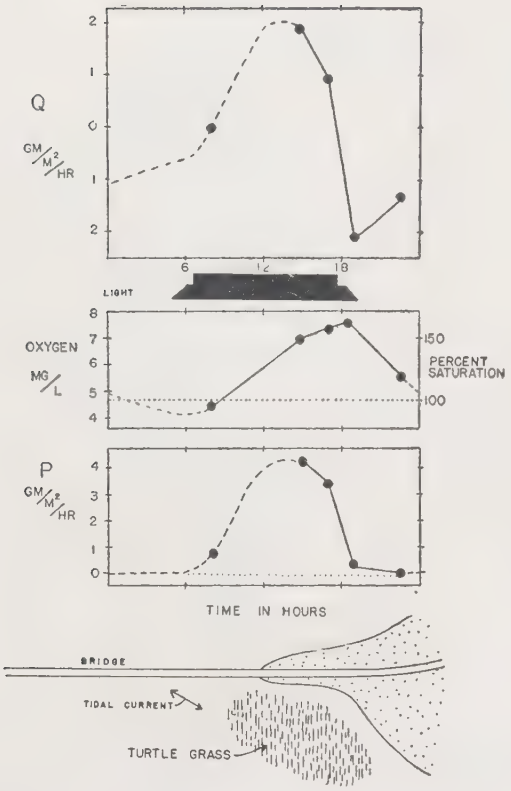

FIG. 11. Rate of oxygen change (Q) and primary production (P) on an area basis in masses of water moving over beds of turtle grass along the causeway on Long Key, Florida, August 14, 1955. The oxygen curve (middle graph) is used to correct for diffusion loss during the day so as to derive the production curve (P) from the oxygen rate-of-change curve (Q). Parcels were followed with fluorescein dye by a wading observer. Points are the mean differences between duplicate samples (Table 3). Current velocity varied from 0 to 0.22 m/sec.

TABLE 2. *Oxgyen changes (mg/L) near moving dye spots over* Thallassia *grass flats Aug. 14, 1955, Long Key, Fla.*

Time of start	Time lapse in minutes	Depth m	Oxygen at start	Oxygen at end	Oxygen change	Rate of oxygen change gm/m²/hr
2:35 pm	7	0.9	6.85	7.05		
			6.81	7.10	0.25	1.86
3:56 pm	14	0.9	7.24	7.49		
			7.20	7.44	0.25	0.96
6:41 pm	5.5	0.9	7.79	7.80		
			8.04	7.62	−0.21	−2.1
10:24 pm	10.4	0.75	5.67	5.45		
			5.80	5.40	−0.32	−1.4
7:53 am	4.8	1.05	4.47	4.43		
			4.48	4.52	0	0

An examination of the carbon-dioxide, nitrogen, and phosphorus values in Table 1 suggests no correlation with the primary production. That the nutrients which limit production under transient conditions are not important in steady state seems reasonable since the large and continuous flows are continually renewing the aquatic medium so that no real deficit of nutrients can develop. If nutrients are not limiting, one next looks to light as the limiting factor. In previous papers Odum and Pinkerton (1955) and Odum (1956b) presented a theory that in steady state when light is the limiting factor an adaptation for maximum power output (production) is developed at a moderately low efficiency. This efficiency is lower than the maximum possible efficiency. These springs may provide real cases to test the operation of this principle. It is not unreasonable that the springs represent the maximum power adjustment to the available light. The efficiencies may represent the optimum efficiency postulated in the aforementioned theory as a requirement of Lotka's principle and the second law of thermodynamics.

An examination of the data in Table 1 shows that the light reaching the community is the main factor controlling production. In Silver Springs the maximum cloudiness in rainy summer days lowered the primary production by half. Whereas this effect is clearly indicated and the effect of the trees is also clearly apparent in the Beecher and Alachua Blue Springs, the cloud effects and tree effects cannot account for the differences in the productivity of relatively unshaded springs studied on sunny days. An examination of the depths of the water over the plant beds, however, revealed that the springs with maximum production have long streamers of rooted plants that support Aufwuchs within a few centimeters of the surface, such as in Chassahowitzka Side Boil and Homosassa Springs. It also seems possible that the anaerobic springs have lower efficiencies, as in Blue Volusia and Beecher Springs, Welaka.

Starting with an insolation for August 1 of about 7000 kCal/m²/day (Kennedy 1949), some rough estimations of light intensity at

TABLE 3. *Production measurements during July and August*

Community	Date 1955	Clouds	Discharge $\times 10^3 m^3$/day	Oxygen production[a] gm/m²/day	Carbon-dioxide assimilation[a] gm/m²/day	Community photosynthetic quotient by atoms ($O_2/CO_2 \times 1.37$)
Beecher	Aug. 2	broken to overcast, rain	0.44	0.68[e]	1.2[e]	0.9
Blue, Volusia	Aug. 9	scattered cumulus	11.7[b]	5.4[e]	14.1[e]	0.5
Weekiwachee	July 26	broken clouds	10.7[b]	10.7[e]	9.4[e]	1.5
Manatee	Aug. 15	scattered clouds	15.0[b]	19.4[e]	51.7[e]	0.5
Silver	Mean of 3 measurements during July–Aug., 1954–55 (Odum, 1957)		68.0[c]	18.0	17.4	1.4
Green Cove	July 7	broken clouds	0.49[d]	15.5	21.2	1.0
Chassahowitzka Side run	Aug. 3–4	broken clouds	2.7[d]	26.7[e]	28.5[e]	1.3
Blue (*Utricularia*)	July 29	broken clouds	0.66[d]	2.0[e]	35.2[e]	0.8
Blue, Alachua	July 28	broken clouds	3.7[d]	5.2[e]	9.1[e]	0.8
Homosassa	July 19	broken clouds	18.6[b]	63.8[e]	95.0[e]	0.9
Rainbow	Aug. 16	clear	70.0[b]	23.9[e]	18.0[e]	1.8
Long Key	Aug. 14	almost clear	—	34.0	—	—

[a] $\frac{\text{(ppm-hrs from graphs) (discharge)}}{\text{area}}$.

[b] Ferguson *et al.* (1947).

[c] Courtesy, U. S. Geological Survey.

[d] Measured with dye and watch at a cross section.

[e] Includes 10% addition for diffusion difference between day and night.

plant level can be made. The following are the factors used for calculating the light energy reaching the plants relative to the light energy reaching the ground on a clear day. Half is considered of wave length suitable for photosynthesis, ¼ is considered removed for broken cloudiness, ½ is considered removed by 100% heavy tree shading, and about 40% is considered removed for the first meter of water (includes surface reflection and refraction). From the estimates of water depth, cloudiness, and tree shading listed in Tables 1 and 3 and the assumptions stated above, an estimate of the visible light energy reaching the plant beds in each spring at the time of the production measurement was computed. In Figure 12 the production measurements and the computed light intensities are plotted. The distribution of points indicates a rough correlation of gross primary production with visible light intensity as thus estimated. The efficiencies seem to be about 4%, with little sign of decreasing efficiencies at the higher production rates. Ten measurements made in every season in Silver Springs (Odum 1957) showed a similar correlation of production and light intensity at about 5% efficiency. That so many varied communities with high production should have so similar efficiencies under conditions of continuously renewed medium supports the theory of an optimum and moderate efficiency requirement for maximum production under naturally adapted and competitive conditions. These results are similar to the seasonal correlations of production with light in naturally adapted lakes found by Verduin (1956).

The absence of light saturation effect in Figure 12 suggests that these permanently fixed benthic algal communities like pine trees on land (Rabinowitch 1951) are sun tolerant at high light intensities, and thus differ from plankton communities studied (Ryther 1956a). Manning, Juday, and

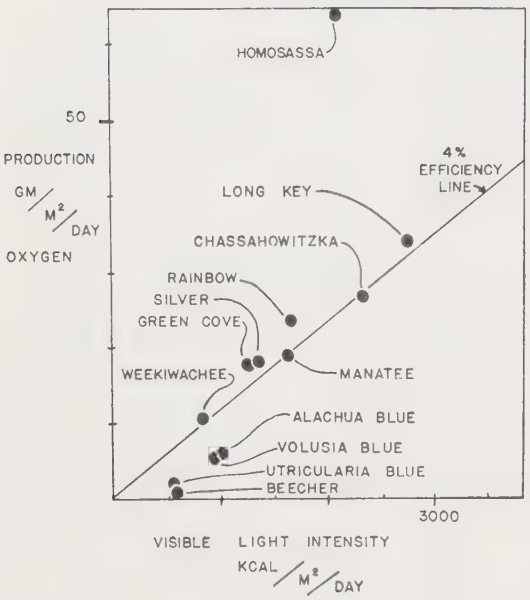

FIG. 12. Gross primary production on an area basis as a function of visible light intensity reaching the level of main plant beds. The production values in Tables 1 and 3 are obtained from areas under oxygen curves in Figures 1–11. A 4%-efficiency line has been drawn on the basis of 3.4 Calories production per gram oxygen released.

Wolf (1938) showed a similar sun tolerance in littoral *Cladophora*.

The range in photosynthesis due to changes in light intensity is apparently greater than that found in plankton communities (Edmondson 1956). The larger range in the spring communities may be due to the absence of a light saturation effect in the normal range of light intensities.

COMMUNITY PHOTOSYNTHETIC QUOTIENTS

The photosynthetic quotients (O_2/CO_2 by atoms) as estimated from the areas under the diurnal curves in Figures 1–10 are presented in Table 3. Like the quotients reported for Silver Springs, the quotients in these varied environments show a correlation with the magnitude of production, suggesting a greater per cent protein production at times of maximum light intensity.

That the quotients are as close to 1 as they are is in one sense a rough confirmation of the order of magnitude of the production values. However, the quotients below 1 are not easily accounted for. Ryther (1956b) summarized data on photosynthetic quotients in populations which resemble those in nature and found that values of about 1.25 were usual. Schmassmann (1951) using diurnal oxygen and carbon-dioxide curves in a Swiss stream obtained a community quotient greater than one.

One possible factor that could lower the community quotient in some springs is a diurnal shift from anaerobic metabolism at night to aerobic metabolism during the daytime, as a result of slight daytime rises in oxygen tension in microenvironments.

Variation in type of nitrogen source can also produce differences in community photosynthetic quotients. As previously suggested on the basis of some nitrate increases observed in two springs, there may be some nitrogen fixation where nitrate-nitrogen is scarce (Odum 1957). More oxygen is released where the nitrogen source is of the nitrate type. Burstrom (1956) summarizes the complex effects on the photosynthetic quotients due to variations in nitrogen metabolism.

HOMOSASSA SPRINGS

The Homosassa Springs is a very peculiar environment. That the enormous primary production in Table 3 is valid seems likely from some large upstream-downstream oxygen increases observed on two other occasions. The area in Figure 9 to the right of the main outflow contains some minor spring inflows which were not included in the computation. If this area had been included, the production per m^2 would have been lower and more like those in the other springs. However, it was visually obvious to the observer that the water passing the downstream station did not include appreciable quantities of water from the minor spring. The strong flow through the long plant tresses marks its passage because the plant blades form stream lines. Thus we must search for some other explanation for the very high efficiency and production in this spring.

The Homosassa Springs is unusual in another respect that may be related to the high production. Large populations of marine fishes mill about in the boil area, where they form the basis of a very fascinat-

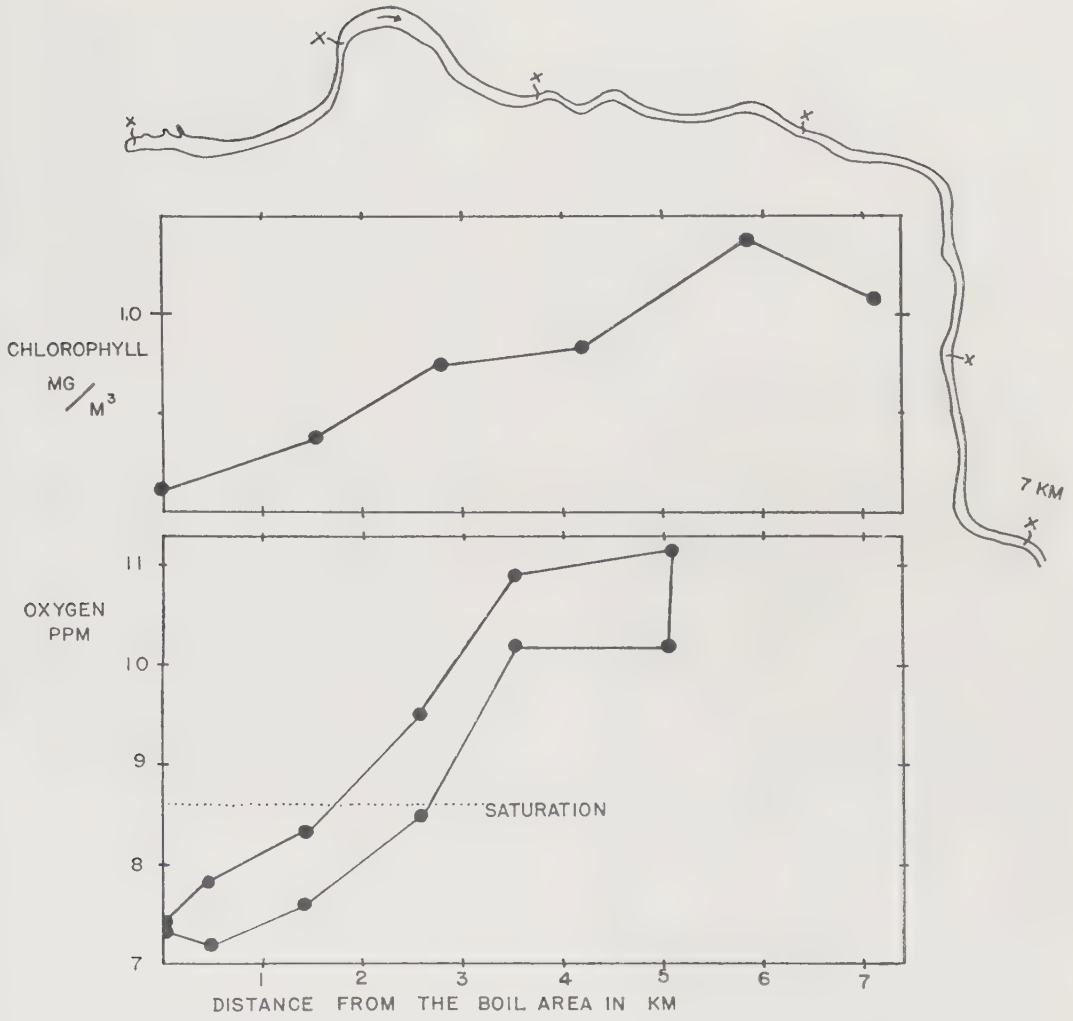

FIG. 13. Downstream increases in chlorophyll and oxygen in the water of Rainbow Springs River. The stations at which chlorophyll samples were made on August 16, 1955, are indicated by *x*'s. Two oxygen samples were made at each station on May 16, 1954, between 1 and 2 p.m. with scattered cumulus clouds. The temperature downstream was 25.5°C. The spring in its upper zones is characterized by beds of *Sagittaria*, shifting to *Chara* and other benthic algae, and visibly increasing turbidity. These data were obtained with Dr. L. J. Marchand.

ing tourist attraction. A cause for the fish concentrations has been previously suggested (Odum 1953). An enormous number of large fishes, far more than are functionally part of the community (possibly 1000) are ever present in the boil. The wastes from these fishes could conceivably provide an unusual nutrient source of heterotrophic energy supplement or important organic requirements. Another peculiarity is the fine, flocculant, reddish, iron precipitate. The water has a green color in gross appearance.

CALCULATIONS BASED ON STEADY STATES IN LONGITUDINAL SUCCESSION

In a previous paper (Odum 1956a) the concept of excess of production over respiration in the spring headwaters was described, with the implication that particulate organic matter was exported from this zone. Fur-

ther downstream the increasing organic content of the water was thought to increase respiration until a balance of production and respiration was achieved. In Figure 13 are plotted a longitidinal series of chlorophyll determinations made in Rainbow Springs River. The boat floated downstream with the water during sampling so that the values obtained represent the change in the water mass in its passage. A suggestion of leveling off at something over 1 mg/m³ chlorophyll is observed 6 km downstream as the character of the river shifts from a predominantly benthic type community to a planktonic one.

Another type of temporary steady-state balance that was observed was the daytime pattern of oxygen from the boil downstream. The water emerging from the boil area during the day receives oxygen at first by production and diffusion. Then as the water attains and exceeds the saturation value a balance develops between production, respiration, and loss by diffusion. This is reached on a sunny day about 5 km below the boil area as observed in Figure 13. If leveling off of the chlorophyll means that exports balance imports at this station, it may be inferred that production balances community respiration at this point also.

The downstream distribution of oxygen on a sunny day in the Rainbow Springs river (Fig. 13) makes possible a rough computation of production, respiration, and diffusion. Consider the zone about 1.8 km downstream from the boil (Fig. 13) where the water is approximately saturated with respect to the oxygen of the air. If the rate of flow is about 0.2 m/sec, the oxygen is increasing at a rate of about 1.2 ppm/hr. Since there is no diffusion in this zone, the following relationship holds:

$$p - r = 1.2 \text{ ppm/hr}$$

where p is the production and r the respiration on a volume basis.

From the chlorophyll graph (Fig. 13) the rate of export of organic matter may be calculated on the assumption that the pseudoplankton has a ratio of chlorophyll to organic matter of 0.002 as in Silver Springs. Twelve hours of photosynthesis

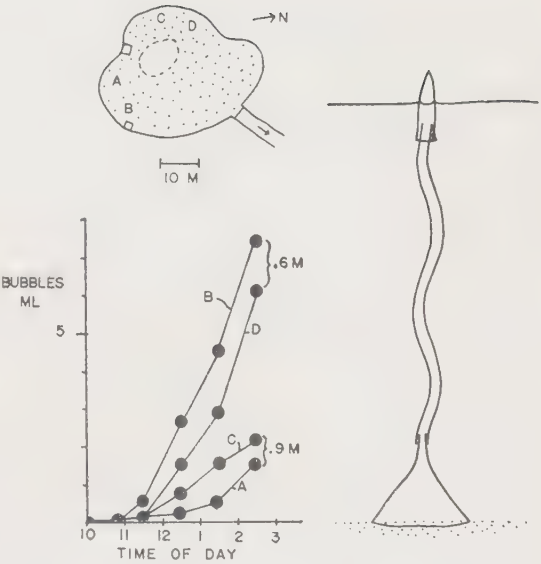

FIG. 14. Bubble production in Orange Springs, Putnam County, Florida. The type of apparatus used to collect bubbles is shown at right. Letters *A–D* indicate locations of funnel apparatus in the map of the springs headwater area at the top. The graph shows the bubble readings at the tip of the apparatus on a sunny day, August 1, 1955. Stations *B* and *D* were at 0.6 m depth; Stations *A* and *C* were at 0.9 m depth.

and 24 hours of respiration are apparently producing an export of 1.4 mg chlorophyll/m³/6 km, as estimated from the upper graph in Figure 13. At a flow of about 0.2 m/sec and a depth of 2 m the export is estimated as 0.17 g/m²/hr dry organic matter. Therefore over a 24-hour period

$$12P - 24R = 24(0.17)\text{g/m}^2$$

where P and R are production and respiration on an area basis. By combining this relation with the equation above inferred from the oxygen graph in Figure 13, R is calculated to be 0.28 g/m²/hr and P 1.48 g/m²/hr during the middle of the day. This value is similar to the approximately 2 g/m²/hr (23.9 g/m²/day) obtained in Table 3 with the diurnal curve method based on Figure 10. The use of data obtained at different times here is justified by the chemostatic nature of the oxygen and chlorophyll conditions. Evidence that chemostatic conditions exist in such springs is given for Silver Springs by Odum (1957).

NET PRODUCTION MEASUREMENTS OF BOTTOM ALGAE IN AN ANAEROBIC SPRING BY MEANS OF BUBBLE ACCUMULATIONS IN FUNNELS

In Orange Spring, Putnam County, Florida, the head pool is partially enclosed by a wall and dam so that the clear anaerobic water emerging from caverns is held temporarily above a sandy bed about one meter below the surface. This sand is a dusky olive-brown due to a heavy film of *Cladophora*, *Gomphonema*, *Lyngbya*, and *Microspora* (Whitford 1956). As shown in Figure 14, funnels (230 cm^2 in area) were placed upon the bottom ooze with extension tubes to the surface terminating in graduated centrifuge tubes. Four such funnel devices were placed on the sand at 10:00 a.m. on August 1, 1955. During the first two or three hours the oxygen produced dissolved in the 275 cc of water in the funnels, and no bubbles appeared. By 1:00 and 2:00 p.m., however, a steady release of bubbles was occurring. The curves of Figure 14 for water 0.6 m deep indicate a rate of gas release 5 times that at 0.9 m depth. The water initially contains about 1 ppm H_2S and 1 ppm free CO_2. The rate of gas release into the shallower funnels in sunlight was 0.15 $g/m^2/day$, which is similar in magnitude to the low estimates for the two other anaerobic springs reported in Table 3. Thus the several methods for measuring in situ community production in nature yield similar estimates.

These measurements establish the range and magnitude of primary production in streams under unusually well defined conditions. It is hoped that many more such measurements in the future may help determine which are the main factors controlling energy flows in trophic systems.

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On the Occurrence of Free Sugars in Lake Sediment Extracts

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ABSTRACT

A semi-quantitative method is described for the determination of free sugars in lake sediments. The method involves extraction with 70% ethanol, deionization with ion exchange resins, and separation and estimation of the sugars on paper chromatograms. The following sugars were detected in extracts of sediments from three Ontario lakes: maltose, sucrose, glucose, fructose, galactose, arabinose, ribose, xylose, and two unknowns. Total amounts of free sugar ranged from traces up to 2.9 g/kg of sediment ignitable matter. Maltose and glucose were usually the dominant sugars. Analyses of two surface cores of mud revealed decreasing concentrations of free sugars from the mud surface down to a depth of 50 cm. The sedimentary sugars are quantitatively held by the mud particles, and are lacking in the pore water of the mud. Lake mud sorbs small amounts of sugars from dilute aqueous solution, but the problem of sorption requires more detailed investigation before its importance can be assessed. Analysis showed that tendipedid larvae were not the source of the mud sugars, and theoretical arguments were presented to show that neither were the living bacteria in the sediment.

Three samples of seston contained 2.3, 3.9, and 42.4 g of total sugar per kg dry weight respectively, with glucose and maltose present in the greatest amounts. The concentration of free sugars in seston decreased by over 90% during aerobic decomposition in the laboratory. Seston is considered to be the main source of the sedimentary sugars, both by directly contributing free sugars to the mud as well as by producing starch-like polysaccharides which can be hydrolysed *in situ* to produce free sugars.

The results of a culture experiment with lake mud showed complete disappearance of sedimentary sugars after 24 days of anaerobic culture in the presence of Bacto-Yeast-N-base. Without the addition of Bacto-Yeast-N-base, more sugar was found after 24 days than was initially present, both in aerobic and anaerobic cultures. Some factor in the Bacto-Yeast-N-base enhanced the disappearance of sugars from mud.

INTRODUCTION

It has been generally assumed by hydrobiologists that if simple organic nutrients such as amino acids and sugars exist in the non-living part of the aquatic environment, the amounts must be vanishingly small. The main basis for this assumption is that both freshwater and marine microorganisms are able to remove the slightest traces of these compounds from dilute nutrient solutions in the laboratory (ZoBell and Grant 1943). Only a few workers have attempted to test the assumption by a direct analysis of the environment. Peterson, Fred, and Domogalla (1925), who examined the dissolved organic matter of lake water for proteins, peptides, and amino acids, were the first to use sensitive analytical methods in an attempt to test the validity of the above assumption. While their data suggested that free amino acids were present in lake water, the method of demonstration was somewhat indirect, and the suggestive evidence cannot be rigorously accepted without more positive proof.

Vallentyne and Bidwell (1956) reported that free sugars were present in 70% ethanol extracts of lake sediments, the total amounts ranging from traces up to 1 g/kg of ignitable matter in the dry mud. Although these amounts may seem small, one should realize that the concentrations greatly exceed the lowest concentrations of sugar that can be utilized by bacteria. The data therefore appeared to contradict the age-old assumption of low nutrient concentrations in

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the non-living environment. The problem seemed sufficiently enticing to warrant further study.

The work reported here was first supported by the Research Council of Ontario and later by the National Research Council of Canada. Grants from the McLaughlin Science Fund of Queen's University permitted continuation of the work during winter sessions.

MATERIALS AND METHODS

Sampling

Sediments were collected from three lakes in eastern Ontario: Lake Opinicon, Little Round Lake, and Upper Rock Lake. Lakes Opinicon and Upper Rock are located near the Queen's University Biological Station at Chaffey's Lock. Lake Opinicon is a shallow eutrophic lake with a maximum depth of 10 m, while Upper Rock Lake is oligotrophic with a maximum depth of 43 m. Little Round Lake, located near Maberly, is oligotrophic with a well developed thermocline in the summer, and a maximum depth of 16 m.

Bottom sediments were collected from these lakes at different times during the summer with a 9-inch Ekman dredge. The dredge samples were mixed until homogeneous before 500 ml aliquots were removed for analysis. The aliquots were mixed with 95% ethanol (usually immediately after collection) so as to adjust the final ethanol concentration to 70%. Toluene was added to each sample as an additional preservative. The samples were stored at 4°C until analysed.

Core samples of mud were collected with the sampler described by Brown (1956). This sampler permitted the collection of an undisturbed profile down to 50 cm depth below the mud-water interface. In order to obtain sufficient material for analysis, about six adjacent profiles were collected. Each profile was separated into 5 cm depth intervals and the samples from identical depths were pooled. Core samples were preserved in the same way as dredge samples.

The mud used for the sorption and culture experiments was collected from the uppermost four inches of dredge samples from Lake Opinicon.

Extraction and concentration

The suspension of mud in 70% ethanol was boiled (about 80°C) for five minutes on a water bath and then filtered through Whatman # 50 filter paper while the extract was still hot. After the filtrate had cooled it was filtered again to remove any material precipitated on cooling. The extracts were deionized to remove inorganic materials (which cause streaking on the paper chromatograms). Amberlite ion-exchange resins IR-120 and IR-4B were used in their hydrogen and hydroxide forms, respectively. Before the resins were first used, they were exhausted and regenerated several times with 10% HCl and 4% NH_4OH, and then washed free of detectable acidity or basicity. Fifty ml of each resin (in glass columns 15 mm in diameter) was sufficient to deionize the extracts with a reasonable margin of safety. The ethanol extracts were passed in series first through the IR-120 column and then through the IR-4B column. The solutions were not basic at any time. The resins were regenerated after each sample had passed through. Although probably unnecessary (and perhaps undesirable as well) the columns were refilled with fresh resins after they had been used for four samples. It was found unnecessary to deionize 70%-ethanol extracts of seston and tendipedid larvae.

The extracts were concentrated by evaporation to dryness *in vacuo* at temperatures under 45°C. The residue was suspended in a measured volume of water (0.5 to 2.0 ml depending on the consistency of the residue). Glass beads were added and the flask shaken so as to break up the residue. As much of the suspension as possible was transferred to a 15 ml centrifuge tube to centrifuge down the particles. Up to one-quarter of the suspension adhered to the walls of the flask and could not be transferred without diluting the concentrate by rinsing. This loss was of no concern, since only aliquots of the concentrate were chromatographed.

Chromatography

Uni-dimensional paper chromatography was used to separate the sugars. A wad of folded filter paper was stapled to the bottom of a sheet of Whatman #1 filter paper in order to increase the flow and evenness of flow during the development of the chromatogram. An equilibrated mixture of butanol, ethanol, and water in the proportions of 45:5:50 (Partridge 1946) was used as the solvent. Chromatograms were developed with the butanol phase for 72 hours. Prolonged development (7 days) was used in some cases. An improved separation of non-pentose sugars results under conditions of prolonged development, but the pentoses are washed off the sheet.

Chromatograms were always run in duplicate. Ten spots (5 to 50 μl in volume) of a standard sugar solution were placed on each chromatogram. The standard solution contained raffinose, maltose, sucrose, glucose, galactose, fructose, arabinose, xylose, and ribose, each in a concentration of 1 μg/μl. Three spots (usually 5, 50, and 100 μl) of the unknown were interposed between the standard spots.

After development, one chromatogram was sprayed with a benzidine spray (Horrocks 1949) which revealed the positions of all the sugars in the standard mixture and their counterparts in the unknown. The other was sprayed with 2% orcinol in 2*N* HCl (Forsyth 1948). The latter spray is specific for ketoses (*e.g.*, sucrose and fructose).

The sugars in the unknown were characterized by (1) their chromatographic positions relative to known sugars, and (2) the colors produced with the spray reagents. A semi-quantitative estimate of the amounts of different sugars was obtained by comparing the color intensities of unknown spots to those of the series of standard spots. The comparison was made (in both visible and ultraviolet light) immediately after completion of the spray reaction. Bidwell, Krotkov, and Reed (1952) found this method to be accurate to within 20%. The accuracy, however, varies inversely with concentration for concentrations up to 40 μg per spot.

The sediment remaining after extraction was dried for 24 hours at 100°C and weighed. The percentage of ignitable matter in the dry mud was determined by ashing over a Bunsen burner for one hour. All sugar concentrations are expressed as mg sugar per kg ignitable matter, except in cases where the percentage of ignitable matter was not determined. The data are actually expressed in terms of a unit weight of 70% ethanol-insoluble sediment rather than a unit weight of sediment before extraction. Since the amount of 70% ethanol-soluble material in most lake muds is low (5% or less) the two values may be taken as equivalent within the limits of experimental error. The dry weights of seston samples and tendipedid larvae were determined before extraction. These materials were dried at 100°C for 24 hours.

DISCUSSION OF METHODS

Sensitivity

The overall sensitivity of the method depends on four factors: (1) the minimum amount of sugar that can be estimated on a paper chromatogram (about 1 μg), (2) the amount of sediment extracted (usually 25 g dry weight), (3) the fraction of the sample spotted on the chromatogram (roughly $\frac{1}{20}$th), and (4) the percentage of ignitable matter in the sample (about 40–50% for the sediments studied). In most cases the minimum amount of sugar detectable was 1 mg/kg dry weight or roughly 2 mg/kg ignitable matter. The presence or absence of sugars can be detected in quantities below 1 μg (using ultraviolet light), but even a semi-quantitative estimate of the amount is impossible. Such quantities will be referred to as "traces".

Efficiency of extraction

Vallentyne and Bidwell (1956) reported that 70% ethanol extracted over 90% of the sediment sugars. We have found, in addition, that duplicate analyses agree within the limits of experimental error. It was also found (late in the investigation) that it is unnecessary to boil the mud-ethanol mixture prior to filtration: cold 70% ethanol extracts the same quantity of sugar from mud as boiling 70% ethanol.

Contamination

The method of analysis is so sensitive that rigorous precautions must be taken to guard against contamination. Several tests were performed to determine the contamination level. A sample of eleven Whatman # 50 filter papers (22.5 cm in diameter) and eleven Millipore HA type molecular filters (4.7 cm in diameter) was extracted with 500 ml of boiling 70% ethanol, and subjected to the same treatment used for sediment samples. No sugar was present in the residue, even though as little as 2 μg in the entire sample could have been detected. When 4 L of double distilled water was reduced to 400 ml, deionized and evaporated to residue, it was found to contain 3 μg of sucrose (*i.e.*, about 1 μg/L) and no other sugar. This amount of sugar is quantitatively insignificant as compared to the quantities present in mud.

These tests show that the sugars present in extracts of lake sediments did not arise from laboratory contamination.

Influence of resins

The advantage of working with deionized solutions is offset by the fact that the resins cause the removal of sugar from percolating solutions. Phillips and Pollard (1953) percolated solutions of sucrose, glucose, and fructose through Amberlite resins IR-120 and IR-400 ('OH). The solutions were unaffected by the IR-120 resin, but the IR-400 ('OH) resin caused retention and degradation. We have carried out tests with Amberlites IR-120 and IR-4B in their hydrogen and hydroxyl forms, respectively. The tests were performed using both aqueous and 70%-ethanol solutions, with 1 mg of each sugar dissolved in 500 ml of solvent. These solutions were "deionized" under conditions identical to those routinely used on mud extracts. The results are reported in Table 1. Losses of pentoses were greatest, followed by hexoses and then disaccharides. Raffinose (the only trisaccharide tested) and fructose were unaffected by the resin treatment. The sugar losses may have been slightly greater for 70%-ethanol solutions as compared to aqueous solutions; however, considering the error involved, the differences are not marked. We have not determined how the disappearance of sugars is affected by the concentration of sugar in the percolating solution, nor did our tests reveal whether the sugars were adsorbed or degraded. The tests were performed using concentrations that might be expected in a sediment extract.

TABLE 1. *Fractional losses of sugars after passage through Amberlite IR-120 and IR-4B ion-exchange columns*

	Raffinose	Maltose	Sucrose	Galactose	Glucose	Fructose	Arabinose	Xylose	Ribose
70% ethanol Old resin	.0	.1	.1	.6	.4	.0	.8	.5	.6
70% ethanol New resin	.0	.2	.1	.8	.6	.0	.9	.7	1.0
Distilled water Old resin	.0	.2	.2	.4	.4	.0	.4	.4	.4
Distilled water New resin	.0	.0	.2	.2	.2	.0	.6	.6	.6

When one considers that the overall reproducibility of the method is about 20%, it is perhaps permissible to overlook the disaccharide losses, but the losses of hexoses and pentoses are more serious. No attempt has been made to correct the data for any of these losses. The values for all sugars therefore represent minimal quantities.

The resin losses of the non-pentose sugars may be less for mud extracts than for pure sugar solutions. In one experiment we achieved quantitative recovery of sucrose, glucose, and galactose when 500 μg of each sugar was added to a mud extract before deionization. Three-fifths of the added ribose, arabinose, and xylose was lost under the same conditions. Maltose was not tested. This suggests that there may be 70% ethanol-soluble materials in mud which reduce the sugar retaining or sugar destroying power of the IR-4B resin.

The possibility that free sugars are produced by polysaccharide hydrolysis on the resins seems rather unlikely under the conditions of analysis; however, it has not been directly tested. It should be stressed that the first analyses for free sugars in sediment extracts were performed without

the use of resins (Vallentyne and Bidwell 1956).

Identification of sugars

During the course of the study, eight sugars were tentatively identified in extracts of lake sediments: maltose, sucrose, glucose, fructose, galactose, arabinose, xylose, and ribose. An unknown sugar, occupying a position near raffinose, occurred in most mud extracts. Since its identify is still uncertain, it will be referred to as "near-raffinose".

As mentioned above, the sugars were characterized by chromatographic positions and color reactions to the spray reagents. The benzidine spray produces slightly different shades of brown with different sugars, thus offering an additional means of characterization. The orcinol reaction was given both by standard sucrose and fructose as well as their sedimentary counterparts, thus adding greater certainty to the identification of these ketoses in sediment extracts.

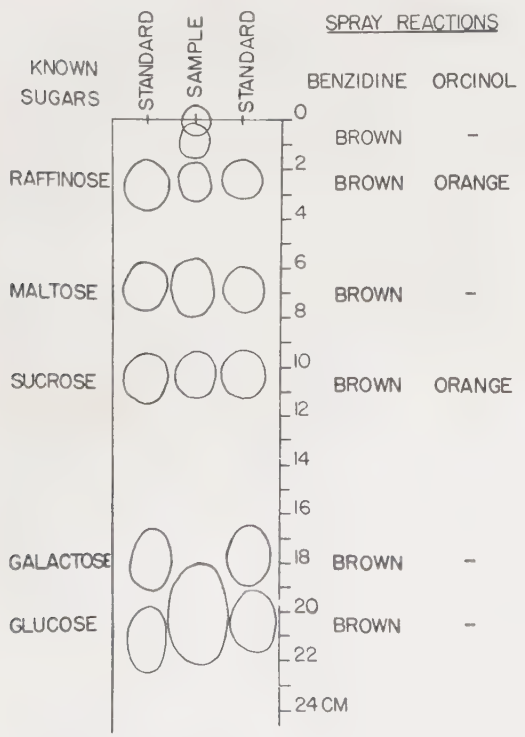

FIG. 1. Tracing of the upper part of a chromatogram which had been developed with butanol-ethanol-H_2O (45:5:50) for 72 hours. The unknown spot in the raffinose region did not react with the orcinol spray reagent.

The comparability in chromatographic position of knowns and unknowns is shown in Figure 1. This is a tracing of the upper part of a chromatogram which had been developed for 72 hours. The lower half of the chromatogram, containing fructose and the pentoses, is not shown.

Free maltose is not a common constituent of living organisms. More thorough tests were therefore used to establish its presence in mud extracts. Co-chromatography of "mud maltose" with an authentic specimen of maltose resulted in no separation using the butanol-ethanol-water solvent under conditions of prolonged development. Paper chromatography with (1) water-saturated phenol and (2) butanol-acetic acid-water (in the proportions of 2:1:2) also failed to separate "mud maltose" from authentic maltose. The only other common sugar running near maltose on paper chromatograms is cellobiose. Cellobiose was co-chromatographed with "mud maltose" using each of the three solvent systems listed above. Separation was achieved only with the phenol solvent. With the phenol solvent, "mud maltose" ran as a single spot which was identical in position with known maltose. The same was found to be true for maltose derived from seston. Although only a few sediment samples were tested in this way, there is clearly the suggestion that the "mud maltose" behaves as a single sugar, which is identical with maltose, and not contaminated with cellobiose.

Maltose is 1,4-α-glucosido-glucose. Schwimmer and Bevenue (1956) have described a reagent mixture which differentiates between 1,4- and 1,6-linked sugars. When this test was applied to "mud maltose" the characteristic purple color of 1,4-linked sugars was produced, thus distinguishing the "mud maltose" from isomaltose (1,6-α-glucosido-glucose). There is little doubt then that the sediment extracts do indeed contain maltose.

The unknown referred to as "near-raffinose" (see Fig. 1) did not react positively to an orcinol spray. Since raffinose (by virtue of its fructose component) does react

TABLE 2. *Sugar determinations on dredge samples from Lake Opinicon, Little Round Lake, and Upper Rock Lake, Ontario*

Samples taken with a 9-inch Ekman dredge. Concentrations are given as milligrams sugar per kilogram ignitable matter. Those values marked (*) are expressed as mg sugar per kg dry weight.

	Date	Maltose	Sucrose	Glucose	Fructose	Galactose	Arabinose	Xylose	Ribose	Near-raffinose	Total	% ignitable matter	Sampling depth in m
Lake Opinicon Location A	10/6/54	+	9	71	0	0	0	3	0	—	83	54.3	6
Lake Opinicon Location A	20/6/54	0	10	18	0	0	0	0	0	—	28	59.3	6
Lake Opinicon Location A	21/7/54	100	5	140	9	0	0	0	9	—	263	52.1	6
Lake Opinicon Location B	9/7/54	46	11	57	0	0	0	0	0	—	114	59.8	9
Lake Opinicon Location A	11/6/55	98*	4*	59*	0	0	0	0	0	+	161*	—	6
Little Round L. Location 1	28/6/54	0	36	36	43	0	0	0	0	+	115	39.5	16
Little Round L. Location 2	28/6/54	0	7	14	0	1	1	t	0	—	23	40.8	16
Upper Rock L.	28/6/54	0	1*	2*	0	t*	0	0	0	—	3*	—	43
Lake Opinicon Dried mud from Location A and Location B	20/6/54 9/7/54	5	12	17	3	0	0	0	0	—	37	59.4	5–9

+ = present, not quantitatively measured.
— = not detected.
t = 0.2–0.5 mg/kg ignitable matter.
0 = less than 0.2 mg/kg ignitable matter.

with orcinol, it will be clear that "near-raffinose" is not identical to raffinose.

A spot reacting with the benzidine spray was occasionally seen in the region between the base-line and the position of raffinose. Since its identity is completely unknown and its purity uncertain, it will not be considered further here.

RESULTS AND DISCUSSION

The results of analyses of dredge samples are reported in Table 2. The data for location A (6 m depth) in Lake Opinicon show pronounced differences, both in the sugars found as well as their concentrations. While it is possible that there may be rapid changes in the metabolism of sugars in mud, it seems more plausible that the differences were either due to the heterogeneity of the mud at the sampling location or else due to variable penetration of the dredge into the mud. The two samples from Little Round Lake, taken on the same day from opposite ends of the lake also showed marked qualitative and quantitative differences, as will be seen in Table 2.

A comparison of the free sugar content of dredge samples from Connecticut lakes (Vallentyne and Bidwell 1956) with the present data reveals a general similarity with two exceptions: (1) maltose was not found in the Connecticut muds and sucrose only once, and (2) galactose was more commonly found in the Connecticut muds. The limited data do not permit a more extensive comparison.

Sediment cores

Deeply buried sediments (7,000–11,000 years old) have a conspicuously lower free sugar content than surface sediments (Vallentyne and Bidwell 1956). It seemed reasonable that the greatest reduction in free sugar content would occur in immediately subsurface sediments. Cores of near-surface sediments were taken from two lakes to determine if this actually was the case. Results for the Little Round Lake

samples are reported in Table 3. Only selected levels were analysed. Analysis of the 25–30 cm interval failed, presumably because the quantity of sediment extracted was too large for adequate deionization. This resulted in streaking of the chromatograms. The data for the Lake Opinicon samples are presented in Table 4. An adjacent series of cores was taken from Lake Opinicon at the same time. The mud (collected from the same depth intervals) was filtered with Whatman # 50 filter paper on a Buchner funnel, then the filtrate was passed through a Millipore HA filter. The pore water was collected from each sample, deionized, concentrated and chromatographed. At the same time the water content of the other set of core samples (used for routine sugar analysis) was recorded. Knowing (1) the amount of sugar in a unit volume of pore water, (2) the amount of pore water in the wet mud samples analysed for free sugars, and (3) the amounts of free sugars in the wet mud samples, one could then compare the distribution of sugars between the pore water and the mud particles.

It will be seen from Tables 3 and 4 that the amounts of free sugar progressively decrease with depth until the total amount of sugar reaches a level of about 0.05 g/kg ignitable matter. This suggests that the sugars are slowly broken down until a comparatively small amount is left when the sediment has been buried to a depth of 20–40 cm. A reasonable guess as to the duration of this breakdown interval (the time necessary for the deposition of 20–40 cm of sediment) is from 20–120 years. During this time there is probably some sort of balance between the supply and decomposition of sugars. With such concentration gradients near the surface, one can quickly appreciate the futility of analysing dredge samples.

Table 3. *Sugar determinations on core samples taken from Little Round Lake, Ontario, August 9, 1954*

Depths refer to the distance below the mud-water interface. Concentrations as grams sugar per kilogram ignitable matter.

Depth in cm	Maltose	Sucrose	Glucose	Fructose	Galactose	Total sugar	% ignitible matter
0–5	.21	.17	.13	.03	.11	.65	51.5
5–10	.04	.02	.06	.01	.00	.13	51.3
15–20	.00	.01	.003	.00	.00	.01	37.8
25–30	—	—	—	—	—	—	36.0
35–40	.00	.01	.04	.01	.00	.06	37.1
45–50	.00	.01	.03	.01	.00	.05	37.5

0 = less than 0.5 mg/kg ignitable matter

Table 4. *Sugar determinations on core samples taken from Lake Opinicon, Ontario, June 28, 1955*

Depths refer to distance below the mud-water interface. Concentrations as grams sugar per kilogram ignitable matter.

Depth in cm	Maltose	Sucrose	Glucose	Nearraffinose	Total sugar	Sugar in pore H_2O, ppm H_2O	% ignitable matter	% water of wet sediment
0–5	1.60	.02	1.30	+	2.92	<0.1	51.9	97.8
5–10	1.00	.01	.90	+	1.91	<0.1	52.1	96.9
10–15	.95	.00	.46	+	1.41	<0.1	51.5	96.2
20–25	.81	.00	.57	+	1.38	<0.1	51.5	95.9
30–35	.34	.00	.17	+	0.51	<0.1	52.5	95.9
40–45	.04	.00	.04	—	0.08	<0.1	54.8	95.9

0 = less than 1 mg/kg ignitable matter

Sugars were not detected in any of the samples of pore water. Calculation shows that in all cases over 96% of the sugar must have been associated with the mud particles, and only 4% or less in the pore water. In the case of the 0–5 cm sample from Lake Opinicon, the corresponding calculation yields values of 99.9 and 0.1%, respectively.

Sorption of sugars on mud particles?

The absence of sugar in the pore water of mud suggested that we might be dealing with some sort of sorption phenomenon. Since the mechanism of binding has so far eluded us, we prefer to use the term sorption, rather than the more commonly used terms adsorption and absorption, referring to sorption at surface and internal sites, respectively.

At first glance, sorption is not a likely possibility because of the non-polar nature of sugar molecules. Lynch, Wright, and Cotnoir (1956), however, have demonstrated that carbohydrates are sorbed on montmorillonite-type clays. The effectiveness of car-

bohydrate sorption on montmorillonite minerals varies directly with molecular weight. The only simple sugar tested by Lynch, Wright, and Cotnoir (1956) was sucrose. It was sorbed to a much lesser extent than oligo- and poly-saccharides. Dr. H. M. Rice of the Canadian Department of Agriculture made X-ray diffraction patterns of two samples of mud from Lake Opinicon, but was unable to identify any of the montmorillonite group of minerals.

If sugars were sorbed on sediments from lake water, one might expect to find dissolved sugar in lake water that is not in immediate contact with mud. Vallentyne and Whittaker (1956) have demonstrated that this is not the case: the highest total concentration of free sugar in four samples of lake water was only 15 mg/m^3, and this was for a sample taken just above the mud surface in Little Round Lake. If anything, there is an enrichment of sugars in lake water adjacent to the mud surface.

On the other hand, the results of a preliminary experiment on the sugar-sorbing power of Lake Opinicon mud clearly showed that small amounts of sugar were removed from aqueous solution by the mud. The sugar concentrations used were greatly in excess over those which we have found to exist naturally, even in the richest surface sediments. Before any conclusions can be reached concerning the sugar-sorbing power of lake muds it will be necessary to obtain data under conditions which more closely simulate those existing in the natural environment.

Sugars in seston

The most direct origin of sedimentary sugars would be from the free sugars present in plankton. Vallentyne and Bidwell (1956) rejected this possibility for all the sedimentary sugars except sucrose, glucose, and fructose, on the grounds that none of the other sugars commonly occurs in the free state in plants. Norris, Norris, and Calvin (1955) reported that small amounts of maltose were present in *Haematococcus* and *Spirogyra*, and larger amounts in *Fontinalis*, but these appear to be exceptions to the rule.

TABLE 5. *Concentrations of free sugars in seston collections from Lake Opinicon, Ontario*

Concentrations are given as g sugar per kg of dry seston.

Sample	Date collected	Maltose	Sucrose	Glucose	Fructose	Total free sugar
Net seston from open water	1/9/55	3.9	1.6	35.5	1.4	42.4
Floating seston	29/6/55	1.0	0.1	1.2	0.0	2.3
Floating seston	17/8/55	1.3	0.0	2.6	0.0	3.9
Decomposed seston (after four months aerobic decomposition at room temp. in the dark)	6/6/55	0.00	0.06	0.06	0.06	0.18

Since there were no available data on the free sugar content of seston, four samples of seston were subjected to analysis. One was a sample of net plankton collected from the open water of Lake Opinicon. Two others were collected from masses of floating algae (mostly blue-greens) which had been blown by the wind into a bay of the same lake. The fourth sample analysed was a collection of floating seston after it had stood in the laboratory in an open bottle (room temperature in the dark) for a period of four months. The results are reported in Table 5. The samples of floating seston were markedly lower in sucrose, glucose, and fructose than the sample of net seston. The laboratory-decomposed floating seston was low in all sugars. The samples of floating seston contained about the same total amount of free sugar as the sample of surface sediment from the Lake Opinicon core, but the open water seston had a value ten times greater (mainly due to the high glucose content). The floating seston was probably more decomposed than the open water seston, as evidenced by the decomposition odors prevalent in the bay at the times of collection. The sequence from net seston to floating seston to laboratory-decomposed seston doubtless reflects the general pattern of sestonic sugar destruction in the lake water. The reason for the lack of maltose in the decomposed seston is not clear. The original collection was unfortunately not analysed while fresh.

The presence of maltose in seston was

unexpected. We are inclined to think that the sestonic maltose originated from the breakdown of starch- and glycogen-like polysaccharides in dead sestonic cells. (It is possible that some of the maltose may have arisen by laboratory breakdown during the process of drying at 100°C.) If a polysaccharide origin is accepted for maltose, it is equally acceptable for glucose as well. One indication is clear: that the free sugars of both seston and sediments may partly originate by the hydrolytic breakdown of polysaccharides. One must consider polysaccharide breakdown to begin with the death of the algal cell, and to continue while the cell remains suspended in the water and even after it has become incorporated into the surface mud.

On the basis of a sestonic origin, sedimentary sugars may be divided into three classes: those which could only originate through polysaccharide breakdown (maltose, galactose, and probably the pentoses as well); those which could originate both by polysaccharide breakdown or directly from the free sugars of living plants (glucose and fructose); and finally those whose only reasonable origin is directly from the free sugars of living plants (sucrose).

Sugars in benthos

The possibility that the sedimentary sugars originated by extraction of the benthic biota has not yet been considered. We may consider two groups of organisms: tendipedid larvae and bacteria. Let us first dispose of the tendipedid larvae as a possible source of the sedimentary sugars. Occasionally, there were as many as four tendipedid larvae present in the mud samples analysed for free sugars. Twelve larvae were extracted and analysed by the same procedure as used for mud samples, except that the extract was not deionized. The twelve larvae contained a total of 20 μg of glucose. No other sugar was present. This amount of glucose is insignificant as compared to the amounts usually found in mud extracts. The larvae were treated as though they had been in a mud sample. Maceration of the larvae might have led to the extraction of more sugar.

The possibility that mud bacteria could be the only source of sedimentary sugar can be quickly eliminated, although admittedly by indirect means. We have been unable to find any published material concerning the presence of free sugars in bacteria. Dr. R. G. S. Bidwell (personal communication) has informed us that he has been unable to detect any free sugar in bacteria, using more sensitive methods than we have had at our disposal. It would appear then that sedimentary bacteria are not a likely source of the sugars.

There is another way in which the question of bacterial origin of the sugars can be answered: by comparing the weight of bacteria to that of sugars in a unit weight of mud. Cooper, Murray, and Kleerekoper (1952) recorded a maximum of 4×10^6 culturable bacteria per g of wet mud in an extensive series of bacterial analyses of the sediments of Lake Lauzon, Quebec. If we assume that the wet mud was 98% water, the maximum bacterial count would amount to 200×10^6 culturable cells per g dry weight of mud. To be on the safe side, we shall multiply this value by five and (in the absence of primary data) use 10^9 cells per g dry weight as the hypothetical bacterial counts for mud from Lake Opinicon and Little Round Lake. This value is equivalent to 10^{12} bacteria per kg dry weight.

Using 5×10^{-11} mg as the average weight of a sediment bacterium (Fox, Isaacs, and Corcoran 1952), calculation shows that the hypothetical maximum weight of bacteria in the sediments studied would have been 50 mg/kg dry weight of mud, or approximately 100 mg/kg of sediment ignitable matter. Thus for any sugar present in a concentration greater than 100 mg/kg ignitable matter, there would be a greater weight of sugar in the mud than bacteria. Even if the total sugar concentration were as low as 10 mg/kg ignitable matter, the bacteria would have to contain at least 10% free sugar in order to completely account for the sedimentary sugar. Most of the sediment samples analysed had total sugar concentrations greater than 10 mg/kg ignitable matter. One must conclude (if the assumptions are correct) that the sedi-

mentary sugars are not totally present in living bacterial cells. Aquatic fungi have not been considered. It is conceivable that some of the sugars were extracted from living fungi in the sediment, but again rather unlikely because of the quantities of sugar involved. On the whole, we can say that some (perhaps all) of the sugars in samples with a very low sugar content may have been derived by the extraction of living benthic organisms, but such a source is quantitatively out of the question for mud samples rich in sugar.

Polysaccharide hydrolysis

We have already indicated that seston (in the most part consisting of plankters, living and dead) is the most probable source of the mud sugar, contributing the free sugar either directly or by polysaccharide breakdown. The hydrolysis of polysaccharides in sediments could be brought about in any one of three ways: (1) attack by autolytic enzymes in the plankton cells, (2) enzymatic attack by sediment microorganisms, or (3) attack by free enzymes in the mud (liberated from dead cells). Kreps (1934) suggested that free enzymes may be important in the metabolism of bottom sediments, and their presence in sediments has been indicated by ZoBell (1939) and Messineva (1940). It must be noted that none of these so-called free enzymes has even been partially purified, so the question of free enzyme attack cannot be taken too seriously without further supporting evidence.

Mud cultures

An experiment was designed to determine the effects of increased bacterial activity on sedimentary sugars. It was thought that this experiment might lead to a better understanding of the state of the free sugars in the mud, particularly on their susceptibility to bacterial attack.

Aliquots (500 ml) from a thoroughly mixed sample of surface mud from Lake Opinicon were placed in six flasks. Two of the aliquots were used as blanks: one was treated in the usual way (see the section on methods) while the other was dumped into boiling ethanol in order to eliminate any possibility of enzymatic hydrolysis during storage. Two other aliquots were mixed with 750 ml of distilled water containing Bacto-Yeast-N-base (Difco)[3]. The concentration of the Yeast-N-base was 300 mg per 750 ml of distilled water. The Yeast-N-base was added to determine if bacterial uptake of free sugar might be limited by low concentrations of non-carbohydrate materials in the mud. The two remaining aliquots were each mixed with 750 ml of distilled water alone.

Two flasks (one with Yeast-N-base and the other with distilled water alone) were flushed for 30 minutes with propane, then for an additional 30 minutes with nitrogen. These flasks were stored in the dark at room temperature for 24 days. The flasks were shaken several times daily to avoid inhomogeneity. The other two experimental flasks (one with Yeast-N-base and the other with distilled water alone) were continuously aerated at room temperature in the dark for 24 days.

At the end of the experiment the pore water was filtered off from each sample and the sediment extracted with eight volumes of 95% ethanol. The extracts of all six samples were deionized and concentrated to dryness *in vacuo*. The results of the

TABLE 6. *Results of an experiment to measure the effects of bacterial action on sediment sugars*

Quantities given as milligrams sugar per kilogram dry weight of sediment. See text for explanation.

Samples	Maltose	Sucrose	Glucose	Total sugars	pH of H_2O at end of experiment
Blank (routine)	4	2	9	15	—
Blank (boiling alcohol)	6	2	9	17	—
Air-Dist. H_2O	31	1	16	48	4.3
Air-Yeast-N-base	10	1	6	17	5.4
N_2-Dist. H_2O	55	9	38	101	6.9
N_2-Yeast-N-base	0	0	0	0	7.0

0 = less than 0.5 mg/kg dry weight

[3] Bacto-Yeast-N-base is a carbohydrate-free mixture of amino acids, vitamins, trace elements, and inorganic nutrients with a composition defined by Wickerham (1951).

experiment are reported in Table 6. The two blanks showed good agreement, indicating that the free sugars in the extracts were not produced by enzyme action during storage in cold 70% ethanol. The flasks with the Yeast-N-base supplement had lower amounts of free sugar than their distilled water counterparts. This suggests that one or more factors in the Yeast-N-base enhanced the bacterial uptake of sugar. Note also that there was an increase in the free sugar content of the distilled water flasks *over and above the amount of free sugar initially present.* Not only was bacterial utilization of sugars hindered in these flasks, but there was an actual production of free sugar during the experiment. It is unlikely that this production was due to a synthesis by microorganisms, for one would have expected this to occur (if at all) in the flasks with the Yeast-N-base supplement. The only datum against this interpretation is the increase of sucrose in the N_2-distilled water flask (if we are correct in thinking that the only source of sucrose is from the free sugars of living organisms). The presence of increased amounts of maltose in the distilled water flasks can hardly be accounted for in any way other than by polysaccharide hydrolysis. As a final observation, note that both the production and utilization of sugars were greatest under anaerobic conditions.

From the data presented above, it is clear that sedimentary sugars can be utilized under favourable conditions. One of these conditions is the presence of one or more non-carbohydrate factors present in the Yeast-N-base preparation. It is conceivable that the *in situ* destruction of sedimentary sugars may be limited by low concentrations of these essential factor(s), thus leading to a pile-up of free sugars produced by polysaccharide breakdown.

CONCLUDING REMARKS

We now feel justified in concluding, on the basis of the data presented here as well as on those previously accumulated by Vallentyne and Bidwell (1956), that free sugars are common constituents of lake sediment extracts. The identification of maltose in the present work has led to the important realization that some of the sedimentary sugars may arise by polysaccharide breakdown. The only alternative explanation is that there are organisms in the mud which synthesize maltose, and this does not appear to be reasonable on other grounds. The hydrolysis of starch- and glycogen-like polysaccharides must occur more readily than that of cellulose, for otherwise we would probably have encountered cellobiose as a breakdown intermediate rather than maltose.

The data obtained from dredge and core samples lead us to believe that there are great horizontal as well as vertical variations in the concentrations of sugars in lake muds. We therefore take great pleasure in chastizing Vallentyne and Bidwell (1956) for their admittedly over-hopeful interpretation of a relationship between sedimentary sugars and seston chlorophyll in four Connecticut lakes—the data were too few. The relationship was probably due to nothing more than chance sampling.

The results of the core analyses are particularly illuminating, for they suggest a comparatively great stability of sugars in lake muds. The cores extended to 50 cm below the mud-water interface, probably representing deposition over 50 to 300 years (100 years is a reasonable guess). It is evident that the half-lives of free sugar molecules in a lake sediment must be at least the order of a few weeks. If shorter half-lives were assumed, one would have trouble in accounting for the presence of free sugars at a depth of 20 cm, for the simple reason that an adequate supply of polysaccharides is not available in the mud to keep pace with so "rapid" a breakdown of sugars. Equally instructive was the demonstration that the sugars were quantitatively associated with mud particles, rather than with the pore water. This datum provides a basis for future work on the state of the sugars in the mud. It also focusses attention on the fact that mud sugars are not free in the sense that they are dissolved in the pore water of the mud. They are free, however, in the sense that they are not components of oligo- or poly-saccharides.

The stability of the sedimentary sugars is probably related to their association with mud particles, but exactly how, we do not know. It is evident that if the mud is supplied with Bacto-Yeast-N-base under anaerobic conditions in the laboratory, the sugars quickly disappear from the sediment. Whether these added nutrients are important in an ecological sense, remains for future experiment.

SUMMARY

1. A method is described for the semi-quantitative estimation of free sugars in 70%-ethanol extracts of lake sediments. The method utilizes ion-exchange resins for deionizing the extracts, and paper chromatography for the separation and estimation of disaccharides and monosaccharides. The validity of the method and criteria of sugar identifications are evaluated.

2. The following sugars were detected in extracts of sediments from three Ontario lakes: maltose, sucrose, glucose, fructose, galactose, arabinose, xylose, and ribose. Two unknowns were also present: one occupying a chromotographic position close to raffinose and the other a position between the base-line and the position of raffinose. The total concentration of free sugars ranged from traces up to 2.8 g/kg of ignitable matter in the mud. Maltose and glucose were usually the dominant sugars. The pentoses (arabinose, ribose, and xylose) were present only in trace amounts; however this may have been partly due to the removal of pentoses by the ion-exchange resins.

3. There was great variability, both qualitatively and quantitatively, in the results of analyses of Ekman dredge samples of mud. This method of sampling is inadequate for the study of sedimentary sugars.

4. Analysis of two sediment cores (down to a depth of 50 cm beneath the mud-water interface) revealed concentrations declining with depth of all free sugars present.

5. The sediment sugars are quantitatively associated with the mud particles, and are lacking in the pore water of the mud.

6. Lake sediments sorb small amounts of free sugars from aqueous solutions, but it remains for future experiments to determine whether sorption is important in the natural environment.

7. Arguments and experimental data were presented to show that the free sugars in sediment extracts are not derived from the free sugars of tendipedid larvae and bacteria living in the sediment.

8. Samples of seston were found to contain larger amounts of free sugars than mud, the values being 2.3, 3.9, and 42.4 g total sugar per kg dry weight for three samples of seston. Maltose, sucrose, glucose, and fructose were identified. Seston decomposed aerobically for four months in the laboratory showed almost complete destruction of free sugars. Seston is considered to be the source of the sedimentary sugar via two paths: by directly contributing free sugars initially present in the seston, and by contributing starch-like polysaccharides which can be hydrolysed to produce free sugar.

9. The results of a culture experiment, with and without added Bacto-Yeast-N-base, indicated a complete utilization of sedimentary sugars under anaerobic conditions in the laboratory in the presence of a Bacto-Yeast-N-base supplement. There was a production of free sugars, over and above the amount initially present, in flasks of mud diluted with distilled water and stored either aerobically or anaerobically for 24 days. One or more factors present in the Bacto-Yeast-N-base preparation favored the utilization of the sedimentary sugar.

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The Vitamin-B_{12} Activity of Mullet and Shark Serum

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ABSTRACT

Microbiological assay procedures using *Euglena gracilis*, *z* strain, were employed to measure the vitamin-B_{12} activity of samples of mullet, shark, and human sera. The average activity was 10.7, 0.84, and 1.15 mμg/ml, respectively. The relatively higher activity of mullet sera compared to shark sera may reflect significant physiological differences between these subclasses of fish and possibly different feeding habits.

Bacteria isolated from mullet intestinal contents were grown in a vitamin B_{12}-deficient medium. Of 8 cultures tested, 6 had vitamin-B_{12} activity when assayed with *Escherichia coli* 113-3 and 1 had activity for *Euglena*. In other experiments concentrates of cell residues were assayed. Two out of 4 supposedly negative vitamin-B_{12} producers had activity for *Euglena*.

INTRODUCTION

The economy of the oceans is influenced to a great extent by the diverse physiological activities of its microscopic communities. Many of these microscopic populations are dependent upon the availability of specific nutrilites or essential growth factors for growth and development. Of those marine microorganisms that have been studied in the laboratory, several diatoms (Hutner and Provasoli 1953, and Droop 1955) and algal flagellates (see list compiled in Provasoli and Pintner 1953) have a requirement for some member of the vitamin-B_{12} family of compounds.

Vitamin B_{12}-active substances are added to the environment as a result of the metabolic activities of various marine microorganisms. Ericson and Lewis (1953) found that 70 per cent of 34 bacterial cultures isolated from marine sources produced vitamin B_{12}-active compounds. Similar data were obtained by Starr *et al.* (1957), although different assay procedures were used. Water movement and other physical factors undoubtedly aid in the dissemination of these products of microbial origin. Thus, these products may influence distant as well as neighboring populations. The suspended matter of sea water with its inherent and/or adhering nutrients contained appreciable quantities of vitamin B_{12} (Starr 1956, and Burkholder and Burkholder 1956). Ericson and Lewis (1953), who assayed the vitamin-B_{12} activity of seaweed, attributed activity to synthesis by bacteria living epiphytically on the algae rather than to the synthesis by the alga itself.

Several investigatiors have reported the presence of vitamin B_{12}-active substances in marine vertebrates and invertebrates. Masaaki (1952) measured and compared the vitamin-B_{12} activity of the organs of fish. He reported also on the presence of vitamin B_{12} in univalves and bivalves (Masaaki 1953). Maxwell (1952) found that molluscs are, in general, richer in vitamin B_{12} than echinoderms, crustaceans, or annelids. Oysters, clams, and to a lesser extent shrimp had appreciable vitamin-B_{12} activity (Robbins *et al.* 1951) suggesting that algal flagellates, an important item in their diet, are either producers or accumulators of vitamin B_{12}-active substances (Provasoli and Pintner 1953).

In this investigation the vitamin-B_{12} activity of mullet and shark sera were measured using microbiological assay procedures. An attempt was made to account for the higher vitamin-B_{12} activity of mullet serum by examining the intestinal bacteria of mullet for their ability to produce vitamin B_{12}-active compounds.

METHODS

The blood samples of 10 mullet and 6 sharks (Table 1) were obtained by cardiac puncture with a glass capillary pipet. In

order to avoid contamination by body fluids, only one puncture was made per fish. Approximately 2 to 5 ml of blood from each fish was transferred into test tubes and allowed to clot undisturbed at room temperature (25°C ± 2.0°C) for several hours. The extruded sera were centrifuged at 8,000 rpm for 5 min at 4°C. Samples were either assayed immediately or stored at −5°C for 8 days prior to use.

For comparative purposes 7 serum samples from man were assayed.

Bacteria were isolated from the intestinal contents of mullet using routine bacteriological techniques. The composition of the medium used for isolation and maintenance of stock cultures has been described previously (Starr and Jones 1957).

The serum samples and bacterial isolates were assayed for vitamin-B_{12} activity using *Euglena gracilis, z* strain, and *Escherichia coli* 113–3. Cultures of these assay microorganisms were obtained through the courtesy of Dr. L. Provasoli and Dr. B. D. Davis, respectively. Except for the dilutions of the serum samples which were made with distilled water, the assays were conducted as described earlier (Starr *et al.* 1957).

RESULTS

The relative vitamin-B_{12} activities of mullet, shark, and human sera are listed in Table 1. The activity of mullet sera ranged from 4.6 to 18.4 mμg/ml (av. 10.7), shark sera ranged from 0 to 2.8 mμg/ml (av. 0.84), and human sera ranged from 0.54 to 2.06 mμg/ml (av. 1.15).

TABLE 1. *Relative vitamin-B_{12} activity (mμg/ml) of sera samples as assayed with* Euglena gracilis, *z strain*

Specimen number	Mullet*	Human	Shark	
1	4.6	0.54	2.81	*Aprionodon isodon*
2	13.4	1.07	1.39	" "
3	13.5	0.73	0	*Scoliodon terrae-novae*
4	6.6	1.20	0.20	" "
5	17.0	2.06	0	*Sphyrna diplana*
6	7.6	1.12	0.63	" *tiburo*
7	18.4	1.32		
8	7.8			
9	7.3			
10	10.7			

* All mullet were *Mugil cephalus.*

TABLE 2. *Vitamin B_{12}-production in mμg/ml by bacterial cultures isolated from mullet intestinal contents as assayed with* Escherichia coli *113-3 and* Euglena gracilis, *z strain*

Culture No.	Aliquots of the same culture assayed by	
	E. coli	*Euglena*
233	5.0	1.9
238	1.0	0 (0)*
249	1.3	0 (0.10)
250	0	0 (0)
255	0	0
259	1.1	0
260	0.4	0
266	1.4	0 (11.9)

* The cell residues of mass cultures numbered 238, 249, 250, and 266 were concentrated by centrifugation and assayed. Their corresponding supernatants had no activity for *Euglena.*

Eight bacterial isolates obtained from mullet intestinal contents were grown in a vitamin B_{12}-deficient medium (Starr *et al.* 1957). Aliquots of the same culture were tested with *E. coli* and *Euglena.* Results showed that 6 cultures had activity for *E. coli* and 1 culture had activity for *Euglena.* However, in experiments designed to determine if vitamin B_{12}-active substances were released into the medium or confined to the bacterial cells, we found that concentrates of the cells of 2 out of 4 supposedly negative vitamin-B_{12} producers had positive activity for *Euglena* (Table 2).

DISCUSSION

The specificity of the available microbiological assay organisms for vitamin B_{12}-active substances is discussed in Ford and Hutner (1955). Briefly, *Euglena* is less specific than *Ochromonas* as it responds to pseudovitamin B_{12} and to factors A, C_1, and C_2, but it is more specific than *E. coli* and *Lactobacillus* (Coates and Ford 1955).

The assay techniques incorporating the *Euglena z* strain were developed by Hutner *et al.* (1956). Their procedures eliminated the excessive alkalinization that caused precipitation of serum proteins during growth of *Euglena.* These investigators stated that the mean normal concentration of human sera is about 0.360 mμg/ml. Mollin and Baker (1955) stated that the serum B_{12} concentrations of normal subjects ranged

from 0.100 to 0.900 mμg/ml. Except for one serum which had an activity of 2.06 mμg/ml, we found a range of 0.54 to 1.32 mμg/ml which is in general agreement with the above values.

The activity of serum samples from mullet and shark ranged from 4.6 to 18.4 mμg/ml and 0 to 2.81 mμg/ml, respectively. The higher values for mullet serum compared to shark serum may reflect significant physiological differences between these subclasses of fish and possibly different feeding habits. Although the information in Table 1 is not conclusive, it suggests that the vitamin-B_{12} activity of shark serum differs between genera and species.

Why or how the mullet accumulates such significantly high and variable quantities of vitamin B_{12} in its serum is unknown. This vitamin does play an important role in the metabolism of animals (Arnstein 1955) and microorganisms (Lascelles and Cross 1955). Although the bacterial flora of mullet intestinal contents may not represent a resident population, the relative percentage of vitamin-B_{12} producers in mullet intestine is about the same as that found in marine muds and waters (Starr *et al.* 1957) and in association with seaweeds (Ericson and Lewis 1953). Mullet may obtain vitamin B_{12} either directly from their food or from their intestinal flora or from both sources.

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The Production of Vitamin B_{12}-Active Substances by Marine Bacteria

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ABSTRACT

The production of members of the vitamin-B_{12} family of compounds by 34 marine bacteria that were grown in a B_{12}-deficient medium was assayed. Using aliquots of the same bacterial culture, 70 per cent and 30 per cent had activity for the assay organisms *Escherichia coli* 113-3 and *Euglena gracilis*, *z* strain, respectively. Using the *Euglena* assay, no apparent relation was evident between the activities of the cell residues or corresponding supernatants and the relative amount of growth of each culture. However, of the 24 bacterial cultures tested, the supernatants of 42 per cent had activity, whereas the corresponding cell residues of 63 per cent had activity.

INTRODUCTION

Extensive reviews are available on members of the vitamin-B_{12} family of compounds dealing with their chemistry, functions in animal and microbial metabolism, distribution, production, and methods of measurement (McNutt 1952, Jukes *et al.* 1954, Hoff-Jørgensen 1954, and Ford and Hutner 1955). Burton and Lochhead (1951) and Lochhead and Burton (1955) have demonstrated that vitamin B_{12} is required by certain soil bacteria. Vitamin B_{12}-active compounds have been shown to be essential or stimulatory for the growth of various marine microorganisms (Hutner and Provasoli 1953, Droop 1954, Lewin 1954, Sweeney 1954, Droop 1955, and Wilson and Collier 1955). Such observations suggest that vitamin B_{12}-active compounds may have a vital role in the economy of the oceans, in production of algal blooms, and in population successions.

In previous studies (Starr 1956), the vitamin-B_{12} content of the suspended matter of estuarine and oceanic waters was assayed. The present investigation was undertaken to learn more about the vitamin-B_{12} cycle in marine environments. Information gained from this and related studies encompassing the vitamin cycles and the roles of organic compounds of the oceans may add materially to our understanding of marine ecology and its application to marine fisheries.

In this investigation microbiological assay procedures were used to measure the production of vitamin B_{12}-active compounds by 34 different bacteria isolated from marine environments. Relative levels of production were compared using two different microbiological assay organisms having different spectra of activity for members of the vitamin-B_{12} family of compounds.

EXPERIMENTAL

The 34 bacteria screened for production of vitamin B_{12}-active substances represent different morphological and physiological groups that are maintained in our permanent stock culture collection. They have not as yet been identified. They were isolated from a variety of marine samples by techniques described in a previous publication (Starr and Jones 1957).

The bacteria were grown in a vitamin B_{12}-deficient medium of the following composition: NaCl, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; KNO_3, 0.02 g; NH_4Cl, 0.01 g; K_2HPO_4, 0.04 g; Na acetate, 0.01 g; sucrose, 0.1 g; glycine, 0.01 g; yeast extract 0.02 g; and distilled water to 100 ml. The pH was adjusted to 7.5–7.7 with 1N NaOH. After 10 days' incubation at 26°C ± 2°C, aliquots of each culture were diluted 1:10 with a buffer at pH 4.5. The buffer contained 8.2 g/L of KH_2PO_4 and 0.5 g/L of $Na_2S_2O_5$. The diluted cultures were hydrolyzed in a boiling water bath for 20 min. After cooling, a second 1:10 dilution was made from the first with the same buffer. These dilutions, 1:10 and 1:100, of each culture were diluted

further with distilled water to give the following final assay dilutions: 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000.

The following procedures were used in order to determine if vitamin B_{12}-active substances were confined to the bacterial cells or released into the medium. Twenty-four of the 34 bacteria were grown in 400 ml of medium in 1000 ml Erlenmeyer flasks. The cultures were incubated at 26° ± 2°C on a rotary shaker for 6 to 8 days. Twenty-ml aliquots of each culture were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was decanted, and final dilutions were prepared from it. The cell residues were suspended in 10 ml of the phosphate-metabisulfite buffer, and final dilutions were made. Other aliquots of each culture were used for turbidimetric growth measurements using a Klett-Summerson colorimeter and for pH measurements.

Samples were assayed for vitamin-B_{12} activity using *Euglena gracilis, z* strain, (Pringsheim's) which was obtained through the courtesy of Dr. L. Provasoli. It is now available from the American Type Culture Collection. The methods and procedures were essentially those of Hutner *et al.* (1956) with minor modifications. Stock cultures of *Euglena* were maintained in screw-cap tubes containing: K_2HPO_4, 2 mg; Na_2·citrate·$2H_2O$, 2 mg; $FeCl_3 \cdot 6H_2O$, 0.2 mg; peptone, 60 mg; trypticase, 16 mg; yeast extract, 5 mg; and made to volume with 100 ml of distilled water. The pH was adjusted to 6.5. Assays were performed in screw-cap tubes (size 125 x 20 mm) using the basal medium, "dry mix", described by Hutner *et al.* (1956). Two and one-half ml of the double-strength basal medium were added to 2.5 ml of each dilution of the sample. Duplicate standard curves were made using commercial vitamin B_{12} to give the following final concentrations (mμg/100 ml): 0.0, 0.05, 0.12, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0. All media were autoclaved at 15 lb for 10 min prior to inoculation. A 6 to 8 day-old *Euglena* culture grown in the stock culture maintenance medium was used for inocula. A bacteriological loop was used to transfer approximately 0.05 ml to 12 ml of the basal medium. One drop of the resulting suspension (approximately 0.08 ml) was used as inoculum per assay tube. Although it has been shown (Østergaard Kristensen 1954) that the supernatant fluid of well-grown *Euglena* cultures contains material which inhibits the growth of *Euglena* in concentrations as low as 1 in 2000 (Hutner *et al.* 1956), our dilution represented a safe margin with a concentration of approximately 1:15,000. Cultures were incubated under a battery of fluorescent lights at 27° ± 1°C for 6 days. Growth was measured using a Klett-Summerson colorimeter.

The other assay organism was the mutant strain *Escherichia coli* 113-3 obtained through the courtesy of Dr. B. D. Davis. The methods of assay were essentially those described by Burkholder (1951) and modified as described previously (Starr 1956). Stock cultures of *E. coli* were maintained on nutrient agar slants and were transferred weekly. Assays were performed as described for *Euglena*. Standard curves were made in duplicate to give the following final concentrations of commercial vitamin B_{12} (mμg/100 ml): 0.0, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 30.0, and 40.0. All media were autoclaved at 10 lb for 3 min prior to inoculation. Inocula were prepared from a 24-hr slant of *E. coli* by carefully transferring a small amount of growth with a fine platinum needle to 12 ml of single strength basal medium. One drop (approximately 0.08 ml) of this cell suspension was used per tube. Experiments were incubated for 18 to 20 hr at 30°C on a tissue-culture apparatus rotating 20 to 24 rpm. Growth was measured turbidimetrically with a Klett-Summerson colorimeter.

RESULTS

Typical assay response curves for *Euglena* and *E. coli* to increasing concentrations of commercial vitamin B_{12} are shown in Figure 1.

The results of the preliminary screen to determine the numbers of bacteria producing vitamin B_{12}-active substances are given in Table 1. Using aliquots of the same culture, 70 per cent and 30 per cent of the isolates

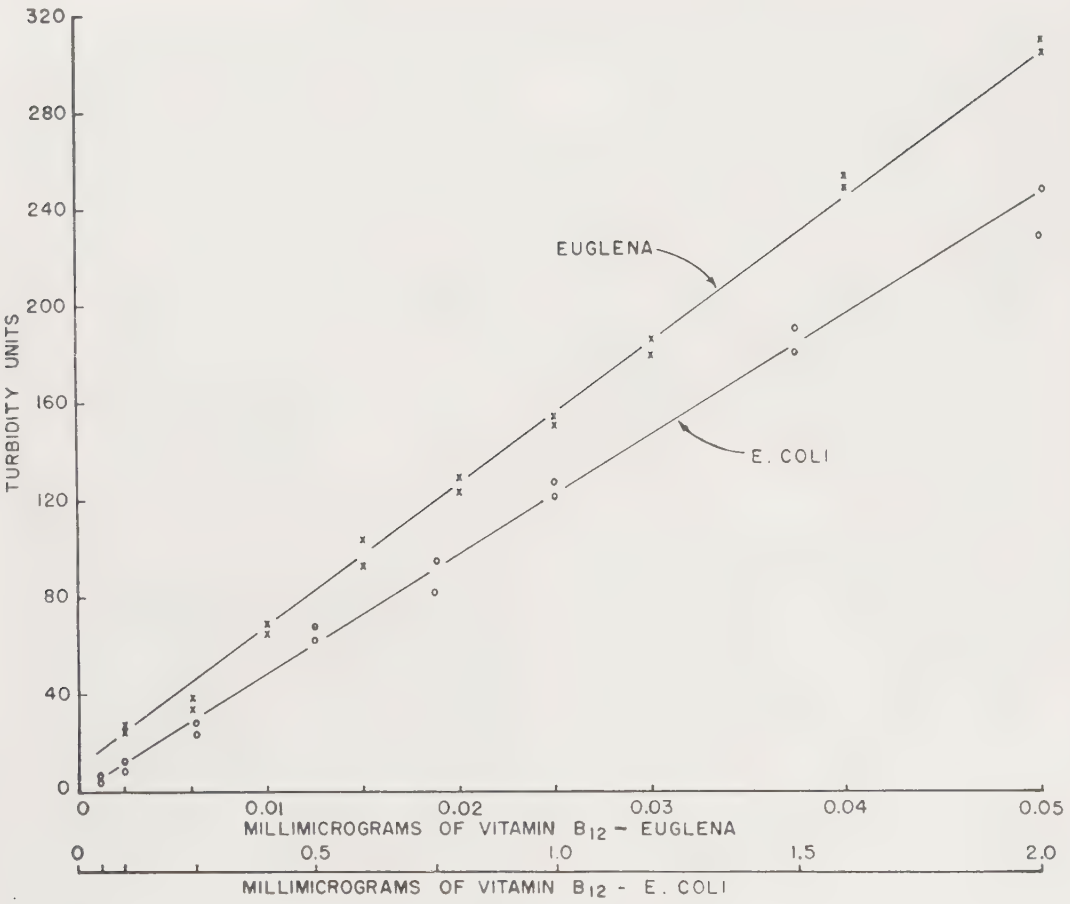

FIG. 1. Responses of *Euglena gracilis*, *z* strain, and *Escherichia coli* 113-3 to increasing concentrations of vitamin B_{12} per 5 ml of medium.

showed activity by the *E. coli* and *Euglena* assays, respectively. In no instance was a culture positive for *Euglena* and negative for *E. coli*.

The relative levels of vitamin B_{12}-activity produced by the different cultures ranged from 0 to 5.0 mμg/ml as measured by the *Euglena* assay and from 0 to 18.4 mμg/ml as measured by the *E. coli* assay.

Other experiments were designed to determine if vitamin-B_{12} activity was confined to the bacterial cells or released into the medium. The cell residues and supernatants of mass cultures were obtained and processed as described. Aliquots of them were assayed with *Euglena*. For comparison, the same cultures used in the previous experiments (Table 1) were screened.

Of the 24 cultures tested, the cell residues of 63 per cent showed activity, whereas the corresponding supernatants of only 42 per cent showed activity (Table 2).

There was no apparent relationship between the amount of growth (turbidity readings) or final pH of each culture and the levels of vitamin-B_{12} activity of either the cell residues or supernatants. For example, the vitamin-B_{12} activity of culture 266 which grew meagerly (turbidity, 15) was confined to the cell residue. Other cultures (126 and 222B) which grew prolifically produced relatively less activity. In no instance did a supernatant have activity and its corresponding cell residue have none, although the reverse situation occurred a number of times.

TABLE 1. *Comparative responses of* Escherichia coli *113-3 and* Euglena gracilis, z *strain, to aliquots of the same bacterial cultures*

Units of activity in terms of mμg vitamin B_{12} per ml.

Culture No.	*E. coli*	*Euglena*
Control	0	0
112	1.2	0.50
116	3.4	1.36
117	3.8	1.38
121	13.0	4.20
122	0	0
126	1.2	0
127A	0	0
127B	0	0
143	1.1	0
144	0.70	0
148	0	0
149	0.98	0
202	1.4	0
203	0	0
205	2.8	1.36
209	0.75	0.24
215	0.70	0
222A	0	0
222B	1.3	0
233	5.0	1.90
238	1.0	0
245	16.7	4.30
249	1.3	0
250	0	0
255	0	0
259	1.1	0
260	0.40	0
266	1.4	0
276	1.4	0.60
279	0.95	0
286	0	0
287	0	0
288	3.4	0.36
306	18.4	5.00

TABLE 2. *Response of* Euglena gracilis, z *strain, to preparations of the supernatants and cell residues of bacterial cultures, and accompanying data on pH and Klett units of growth*

Units of assay activity in terms of mμg vitamin B_{12} per ml.

Culture No.	Klett units	Final pH	Cell residues	Supernatants
Control	0	7.4	0	0
112	107	7.0	7.00	0.048
116	64	8.4	4.50	0.200
117	66	8.2	2.68	0.035
121	35	7.4	16.2	0.550
122	48	8.4	0	0
126	295	6.3	0.16	0
127A	45	8.2	0.02	0
127B	45	8.2	0	0
143	289	6.4	0	0
144	54	6.9	0	0
148	40	5.9	0	0
203	20	8.2	0	0
205	168	6.6	13.3	0.059
209	110	7.3	1.50	0.004
222A	29	8.3	0	0
222B	222	5.8	0.05	0
233	65	6.8	1.88	0.038
238	107	8.0	0	0
249	259	6.7	0.10	0
250	29	8.5	0	0
266	15	7.7	11.9	0
276	215	7.7	14.0	0.055
279	182	5.0	0.21	0.022
306	30	7.5	3.84	0.870

DISCUSSION

The responses of *E. coli* and *Euglena* to members of the naturally occurring B_{12}-vitamins are discussed in detail by Coates and Ford (1955), Ford and Hutner (1955), and Hutner *et al.* (1956). *E. coli* responds to non-specific substances such as methionine and to Factor B, whereas *Euglena* does not respond to either of them. Factor B (Kon 1955) is the non-nucleotide moiety common to several of the B_{12}-vitamins. If this factor or any one of a number of nucleotides are present, some organisms can complete the synthesis of a form of "vitamin B_{12}" containing that particular nucleotide (Coates and Ford 1955). From the standpoint of the requirements of man, the clinically active vitamin B_{12} or cyanocobalamin is of particular importance. From the standpoint of the growth requirements of marine microorganisms, the pseudovitamin B_{12} and related factors may prove to be as important as cyanocobalamin. Various investigators have demonstrated that cyanocobalamin is a growth factor required by some diatoms and flagellates (Provasoli and Pintner 1953). In addition, Droop (1955) showed that the cyanocobalamin required by the pelagic diatom *Skeletonema costatum* was spared by pseudovitamin B_{12}, Factor A, and Factor B. His noteworthy observations on the lack of specificity of the form of vitamin B_{12} preferred by this diatom indicates the probable importance of "cobalamins" (members of the vitamin-B_{12} family of compounds

in addition to cyanocobalamin) in the economy of the sea.

In this investigation vitamin B_{12}-activity produced by marine bacteria was measured with *Euglena* and *E. coli* both of which have different spectra of activity. Of the 34 cultures screened, 30 per cent and 70 per cent had activity for *Euglena* and *E. coli*, respectively. These data are in agreement with those of Ericson and Lewis (1953) who screened 34 cultures using the *E. coli* 113-3 cup-plate assay and found that 70 per cent were producers. Burton and Lochhead (1951) found that 65 per cent of their bacterial cultures were producers. They examined terrestrial isolates using the *Lactobacillus lactis* assay. A table describing the responses of the different assay organisms to vitamin B_{12}-active compounds is given in Ford and Hutner (1955).

In all cases involving aliquots of the same culture which were tested with both assay organisms, the *E. coli* assay gave decidedly higher values (Table 1). This observation indicates the broader spectrum of response of *E. coli* to vitamin B_{12}-active substances compared to *Euglena* and the wide variation of specific vitamin-B_{12} activity produced by different cultures under similar conditions. The maximum levels of vitamin-B_{12} activity produced by culture no. 306 were 5.00 and 18.4 mμg/ml for *Euglena* and *E. coli*, respectively. Using the *L. lactis* assay Burton and Lochhead (1951) found terrestrial bacteria which produced over 500 mμg activity per ml of culture medium. Burkholder and Burkholder (1956) found marine bacteria which produced up to 150 mμg/ml. They used the *E. coli* agar plate method. Using the *E. coli* 113-3 cup-plate assay, Ericson and Lewis (1953) found marine bacteria which produced 0.005–140 mμg/ml. They stated that a level of approximately 10 mμg/ml is more comparable to those obtained for bacteria isolated from seawater and seaweed. This figure is in agreement with the levels (0.40–18.4 mμg/ml by *E. coli*) found during this investigation.

In experiments designed to determine if vitamin B_{12}-activity was confined to the cell residues or released into the medium, results varied with each culture as shown in Table 2. In general intracellular levels of vitamin B_{12} were higher than extracellular. No apparent relationship was evident between the levels of activity of either the cell residues or supernatants and the final bacterial growth or pH of the medium.

As shown in this and related studies by other investigators, the amount and specificity of B_{12}-activity produced by a particular bacterial culture varied with the conditions of the experiment and methods of measurement.

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The Tidal Migrations of *Donax variabilis* Say[1]

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ABSTRACT

The behavior of the beach clam, *Donax variabilis*, resembles that of *Donax semigranosis* in that it migrates up and down the beach with the rise and fall of the tide assisted by the wash of the surf. On the rising tide the acoustic shock of the breaking waves stimulates the bivalve to emerge from the sand in advance of the uprush so that the wash carries it up the beach. On the falling tide it ignores the acoustic stimulus and emerges into the backwash to be carried down the beach. The reactions may be due to an intrinsic rhythm, changes in the water content of the sand, or a trigger-and-memory mechanism.

INTRODUCTION

The beach clam, *Donax*, is a small active pelecypod that lives between the tide lines in the surf-washed zones of sandy beaches exposed to the open ocean. It is one of the few sedentary or semi-sedentary organisms adapted to this precarious environment where there is constant danger of being buried by the shifting sands or of being stranded above the high-trade mark. Other active clams such as *Spissula* and *Mesodesma* live in a similar environment below the low-tide mark, but when storms of exceptional violence force them into the surf zone they either wash up in windrows above the high-tide mark or become buried at a fatal depth. *Donax* on the other hand manages to withstand the action of the surf and sometimes develops incredibly large populations. Coe (1953) described a population of *D. gouldi* on the beach at La Jolla, California, where the average density exceeded 20,000 individuals per square meter in a band 2 to 5 meters wide extending for more than five miles.

The adaptations that permit *Donax* to live in the surf zone do not appear to have been described in detail in the Occidental literature. Pearse *et al.* (1942) reported that *D. variabilis* moved up and down the beach with the rise and fall of the tide at Beaufort, North Carolina, but did not explain how the migrations were accomplished. Hedgpeth (1953) reported information provided by Burkenroad who had observed similar migrations of *Donax* sp. on the sand beaches of the Gulf of Mexico. He indicated that this species accomplished its migrations by popping out of the sand into the surf. On a rising tide the organisms would pop out in front of the uprush to be carried up the beach and on a falling tide they would emerge into the backwash. Hedgpeth also referred to two papers in Japanese by Mori (1938, 1950) which he had not seen. Jacobson (1955) studied *D. fossor* at Rockaway Beach, New York, and came to the conclusion that this species was merely flushed from the sand by the rushing waters to be carried in either direction by the surf. By using the foot and siphons as a drag the organism retarded its motion sufficiently to prevent it from being carried to the full advance of the wash where it would be in danger of being stranded.

In 1953 the present authors made a series of observations on *Donax variabilis* at Wrightsville Beach, North Carolina. The objective of the study was to determine if this species was sorted according to size by wave action in a manner similar to that reported by Turner (1952) for the soft clam, *Mya arenaria*, in Boston Harbor. It soon became apparent that no such sorting took place, but instead specimens of all sizes migrated up and down the beach with the rise and fall of the tide assisted by the wash of the surf. The authors were not aware of the works of Mori or Hedgpeth at that time but later read Hedgpeth's paper. Subsequently,

[1] Contribution Number 886 from the Woods Hole Oceanographic Institution.

copies of Mori's papers were obtained and English translations were made of the pertinent portions of the Japanese text. The behavior of *D. semigranosis* as reported by Mori corresponded closely to that of *D. variabilis* observed by the authors. However, in view of the fact that Mori's papers are not readily available and much pertinent material is in Japanese, there seems to be some justification for reviewing his work and describing the behavior of *D. variabilis* for comparison.

Mori (1938) studied the behavior of *Donax semigranosis* on the beach along the coast of Okinosu, Japan. He reported that this species accomplished its flood-tide migration by jumping out of the sand in advance of the uprush of the surf, thereby placing itself in a position to be transported landward. While riding the surf up the beach the foot was extended as a drag so that the organism never reached the upper limit of the wash. As soon as the velocity of the wash subsided, the foot was inserted into the sand and the organism dug in as the backwash began to retreat. Mori reported that 3 to 6 seconds were required for *D. semigranosis* to dig in completely so that the slower individuals were frequently carried back some distance. He also reported that this activity continued as the tide rose, resulting in the entire population moving landward in the upper part of the surf zone, the distance of migration being as much as 30 meters on the spring tide. Mori also noted that the organisms appeared to anticipate only the larger waves which would effect the longest possible transport; when low or moderate waves broke they remained in the sand. He then concluded that the jumping reaction was a response to the acoustic vibrations in the substratum which were generated with sufficient intensity only by the larger waves. Mori demonstrated this experimentally by generating similar shocks with his foot and by dashing water violently on the shore, both of which evoked the jumping reaction.

Mori also studied the movements of *D. semigranosis* on the ebb tide and determined that at this phase the clams remained below the surface of the sand until the surf had begun to retreat down the beach. They then jumped out into the backwash where they were carried seaward. It is not clear from the available translation whether Mori believed there was any difference in the sequence of physical events at the opposite phases of the tide that influenced the timing of emergence with respect to the wash. Later (1950), however, he made further observations and suggested that *D. semigranosis* might be endowed with an intrinsic rhythm synchronized with the tidal cycle, which caused it to anticipate the uprush on the flood and await the backwash on the ebb.

THE BEHAVIOR OF *DONAX VARIABILIS*

Donax variabilis is a common inhabitant of the beaches of the Middle and South Atlantic Coast of the United States. At Wrightsville Beach, North Carolina, where the observations to be reported were made, the shore consists of fine sand which is so compact that footprints made by a person of average stature are scarcely visible. In September, 1953, the beach sloped 2.7 feet per hundred feet so that the 3.8-foot tide produced an intertidal zone approximately 150 feet wide. Some portion of the intertidal zone was always awash with surf which rolled in from a line of breakers plunging on an outer sandbar some distance from shore. There were generally at least three rows of white surf between the breakers and the shore line. Here the turbulence was so great that the bottom sediments were continually agitated, as a result of which finer particles were continually being washed out, leaving an accumulation of shell fragments and other coarse heavy material. As each line of surf reached the slope of the beach, the flow became smoother and the bottom sediments were scarcely disturbed. The few pebbles and shell fragments on the surface of the sand were continually moving back and forth with the advance and retreat of the wash, eventually coming to rest in the usual windrow of debris above the high-tide mark.

The average extent of the wash zone was approximately 45 feet during the study, although irregular wave patterns frequently produced considerable variation. During

sequences of regular waves the period averaged seven seconds. The underlying sand in the seaward half of the wash zone was saturated at all stages of the tide, while the sand in the landward half was somewhat undersaturated. Since the sand in the seaward half was incapable of absorbing water, a wet slick remained on the surface as each backwash retreated. The sand in the landward half on the other hand could absorb small quantities remaining on the surface so that no slick remained. Thus at the instant that the backwash had retreated to its fullest extent it was possible to divide the wash zone into a saturated or "wet" portion on the seaward side, and an unsaturated or a "dry" portion on the landward side.

The first collections of *Donax variabilis* were made at dead low tide. The majority of the population was concentrated along the middle of the wash zone with a few scattered specimens occurring up the beach as far as the mid-tide line. All collections produced specimens of mixed sizes indicating that no sorting for size had taken place. Continued collections taken during the first half of the flood tide showed that the population was moving up the beach with the tide, but the rapid sequence of events made it difficult to observe how this was accomplished. Then a fortunate combination of circumstances provided a clue to the mechanism of the migration. During a sequence of high, regular waves the backwash of one wave collided with the uprush of the subsequent one and prevented it from overrunning the wash zone in the usual manner. At the same time an unusually large breaker crashed on the outer bar. Immediately a row of *Donax* emerged from the sand, each specimen lying motionless on its side. The row extended along the beach as far as the eye could see. The next uprush then picked up the specimens and carried them landward, and as the velocity decreased each specimen inserted its foot into the sand, tipped up on its anterior extremity, and dug in in a matter of seconds. Closer observations made during subsequent excursions of the wash showed that similar behavior took place whenever a large wave broke. In general the animals emerged into the uprush just as it passed over them, but there was a number of occasions when they anticipated it by emerging beforehand. It was also determined that the foot was extended as soon as the organisms began to ride the surf to retard their velocity and prevent them from being carried to the limit of the uprush.

The coincidence between the breaking of the waves and the emergence of the animals lead immediately to the idea that the acoustic shock generated by the breakers caused the emergence. To test the theory experimentally, a similar shock was generated during a sequence of low waves by stamping on the sand with the foot. When this was done a number of organisms emerged in a circle several feet in diameter. Such experiments continued whenever circumstances permitted demonstrated that the organisms would emerge only from the "wet" or saturated portion of the wash zone and that one or more washes of surf had to pass over an organism before it would repeat the process. Careful observations revealed the normal pattern of behavior to be as follows: As each large breaker generated an acoustic shock, the organisms would emerge from the saturated sand either in advance of the uprush or immediately into it and be carried up the beach where they would quickly dig in. Specimens failing to reach the unsaturated portion of the wash zone would then wait until one or more washes passed over them before emerging again. In this manner the entire population was carried up the beach, finally arriving at the upper, unsaturated portion of the wash zone at full high tide.

As soon as the tide turned the population began to move seaward. The animals accomplished this by waiting until a large wash of surf had passed over them and had begun to retreat down the beach. They then emerged from the sand into the backwash which carried them down the beach. Again each specimen extended its foot as a drag, and dug in rapidly when the velocity decreased enough to permit the foot to obtain purchase in the sand. During the ebbing phase of the tide the organisms

failed to respond to the crash of the breakers or to shocks generated by stamping, but reacted only to water passing over them. The latter reaction was confirmed experimentally by simply splashing a large bucket of water up the beach at an appropriate moment. When this was done the organisms waited for an instant and then emerged from the sand as the water ran back down the beach. This simple experiment performed during periods when the waves were low demonstrated that the organisms reacted to the passage of water over them only if they were in the upper, unsaturated portion of the wash zone. In addition they would react only if a considerable amount of water passed over them, and once transported they would wait until one or more washes passed over before emerging again. In this manner the entire population was carried down the beach with the ebbing tide except for occasional individuals that became stranded.

Observations were continued for a number of tidal cycles and the experiments were repeated whenever the circumstances permitted. The reactions of the organisms to the passage of water over them was tested on the flood tide with negative results. Their reaction to the acoustic shock during the flooding tide was confirmed a number of times. In a like manner the reaction to water and the lack of response to the acoustic shock on the ebbing tide was also confirmed.

DISCUSSION

The results of two independent studies and a third observation leave little doubt that at least two species of *Donax* change their pattern of behavior simultaneously with the change of the tide, resulting in the surf washing them up the beach on the rising tide and down the beach on the ebb. Neither investigation however has revealed the mechanism that caused this change in activity. Mori's suggestion of an intrinsic rhythm synchronized with the tidal cycle is attractive in view of current interest in so-called biological clocks, but it does not seem adequate. Continued survival of the *Donax* populations would depend on nearly perfect synchrony between the activities of the organisms and the cycle of the tide. Slight phase differences might leave the population stranded above the high-tide mark, or transport it into the turbulent surf zone at low tide. Thus it is difficult to see how such perfect synchrony can take place, since the tidal cycle alone is subject to irregularities resulting from meteorological conditions.

Any alternate hypothesis requires that the organisms be capable of sensing some variation between the conditions prevailing during the opposite phases of the tide, or that some stimulus at each extreme of the tide triggers a persistent pattern of behavior. The one obvious difference between the ebb and the flood is the direction of change of the still-water level, which has a maximal rate of less than 0.1 foot per minute. The main effect this slow change has on the organisms is to make their environment become progressively wetter on flood tide and progressively dryer on the ebb. Thus it is possible that the change in moisture content of the sand acts as a controlling mechanism which influences the organism to react to the acoustic shock of the breakers as its environment becomes wetter, and to the rush of the wash as the environment dries out. In order for this mechanism to operate successfully it is necessary for the organism to be able to ignore minor changes in the moisture content produced by sequences of high waves followed by short periods of relatively calm water.

It is also possible that *Donax* may be equipped with a timing mechanism that acts as the controlling stimulus. If this should be the case the acoustic stimulus would affect only those organisms that had been situated in saturated sand for a certain length of time on the rising tide, and on the ebb the stimulus of the wash would affect only those organisms that had been long enough in unsaturated sand. This hypothesis requires that the organism's timing mechanism be accurately tuned to the rate of change of the water level and does not entirely take care of variations resulting from complex wave patterns.

As a final suggestion a trigger-and-memory hypothesis is proposed. This hypothesis

also involves a timing mechanism but a less precise one. It is proposed that conditions that occur at each extreme of the tidal cycle trigger the organism to commence the appropriate behavior pattern for the particular phase and that this pattern persists until reversed by the opposite stimulus. Thus as the tide approaches its full height the organism moving from saturated to unsaturated sand finally arrives at a level where the sand never does become saturated. A somewhat prolonged wait in this unsaturated environment might trigger the organism to ignore the acoustic stimulus and react only to the wash. The organism then "remembers" the trigger stimulus and exhibits the appropriate behavior until it finally comes to rest in sand that remains saturated. This then triggers the flood tide behavior. The trigger-and-memory hypothesis thus offers a possibility of explaining the observed abrupt change in the behavior at the tidal extremes and the apparent invariability of the reactions of the organisms during each phase of the tide.

The different behavior of *Donax fossor* described by Jacobson deserves some consideration in view of the suggestion by Abbot (1954) that this species may only be a cold-water variety of *D. variabilis*. It is obvious from Jacobson's account that the character of the beach at Rockaway is quite different from that at Wrightsville. Jacobson states that the wash becomes so completely roiled with sand as to make observations impossible, indicating that the sediments are not nearly so compact as are those at Wrightsville. He also reports that *D. fossor* migrates only twenty feet. If this distance is the full width of the intertidal zone the beach has a slope of nearly 20 feet per hundred feet in contrast to the slope of 2.7 feet per hundred at Wrightsville. The steep profile of the beach provides a situation in which the wash of the surf would run up the beach in a much shorter time after the wave breaks than on beaches of more gentle slope where it could wash the animals out of the loose sand before they could respond to acoustic stimuli. Thus any similarity of the behavior of *D. fossor* and *D. variabilis* may have been obscured by the physical conditions of the environment at Rockaway.

SUMMARY

The observed behavior of *Donax variabilis* agrees with that described independently by Mori for *D. semigranosis*.

On the flood tide *D. variabilis* responds to the acoustic shock of breaking waves by emerging from the sand in advance of the uprush and rides the surf up the beach. It only reacts in this way if it is situated in saturated sand.

On the ebb tide *D. variabilis* responds to the passage of the wash over it and emerges into the backwash, as a result of which it is transported down the beach. It only reacts in this way if it is situated in unsaturated sand.

Three hypotheses are proposed to explain this unique pattern of behavior.

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Studies of algal antibiosis using *Haematococcus* and *Chlamydomonas*

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ABSTRACT

Five common freshwater algae were grown in the ten possible two-membered combinations. No two species grew as well in mixed cultures as they did separately. One combination, *Haematococcus-Chlamydomonas*, was selected for more detailed study. When grown together in the same container, *Haematococcus pluvialis* and *Chlamydomonas reinhardi* were found to be incompatible. The latter invariably dominated the cultures, usually within 3–5 days after inoculation. The rate at which *Chlamydomonas* eliminated *Haematococcus* was influenced by a number of factors, the most important being light intensity and nitrogen source. Inhibition of *Haematococcus* was due to a fat-like extracellular substance that was soluble in alkaline medium, somewhat heat stable, and steam distillable from an acid medium. The inhibitor was produced by *Chlamydomonas* and liberated upon the death of the cells. It is suggested that the inhibitor is probably a long-chain fatty acid or mixture of such acids.

Eight long-chain, unbranched, saturated, and unsaturated fatty acids of known composition were assayed against six algae. Palmitic was the most toxic saturated acid tested, linoleic the most toxic unsaturated. *Haematococcus* was the most sensitive alga tested, *Chlorella* and *Scenedesmus* the least sensitive. There was a relatively narrow range of concentrations between those causing no inhibition and complete inhibition in the case of *Haematococcus*.

The results of the present study are compared with those from other investigations of algal antibiosis.

INTRODUCTION

In 1931 a novel explanation was advanced by Akehurst to account for the marked fluctuations that normally occur in most algal populations. This explanation was based almost entirely upon the supposition that most algae secrete into the surrounding water extracellular organic compounds that are able to inhibit the growth of some algae and accelerate the growth of others. Unfortunately, at the time he proposed it there were very few data to support Akehurst's contention that algae are able to produce toxic substances. Proof of this point was, however, later supplied by Pratt (1940) who was able to show that *Chlorella vulgaris* produced an extracellular antibiotic substance(s) that limited its own growth. Pratt's work was later verified by Rice (1954). The data of Pratt and Rice showed conclusively that, at least in laboratory cultures, one alga could inhibit the growth of a second, or in some instances the effect was one of mutual inhibition by, and of, the two species.

Somewhat similar evidence along these same lines also has been offered by Flint and Moreland (1946), von Denffer (1948 and 1949), Lefevre *et al.* (1952 and 1956), Harder and Oppermann (1953), Jakob (1954), and Steemann Nielsen (1955). Although there is now a considerable body of evidence to support Akehurst's original hypothesis very little information is available regarding the nature of the inhibiting substance.

The object of the present investigation was to consider in some detail still another case of algal inhibition with the elucidation of the toxic principle as the ultimate goal. Work on the problem was interrupted before the final attainment of this goal, but sufficient data are available to permit a number of generalizations to be made regarding the inhibiting mechanism, and a "summing up" at this point would appear to be warranted.

The work reported in this paper was carried out in the Osborn Zoological Laboratory at Yale University where the author was a fellow in cancer research of the American Cancer Society. The author would like to

acknowledge the many helpful suggestions and innumerable courtesies extended to him by Professor G. E. Hutchinson. Thanks are also due to Joseph Shapiro and R. W. Castenholz for critical reading of the manuscript.

PRELIMINARY EXPERIMENTS

Several preliminary experiments were undertaken to ascertain the effects of one alga on the growth of a second. These experiments consisted essentially of growing the algae separately and in various combinations and comparing the relative number of cells produced in the two cases. The methods used in these investigations were much the same as those previously described (Proctor 1957). Pure cultures of algae, which were used throughout the study, were grown in a constant-temperature light room. The various strains of algae that were used are listed in Table 1. The composition of the different media that were employed in the present investigation is shown in Table 2.

The experiment involving *Chlamydomonas* and *Haematococcus* may be taken as a typical example. Two 125-ml flasks, each containing 50 ml of Medium II, were inoculated with *Haematococcus*, two more with *Chlamydomonas reinhardi*, and two with a combination of *Haematococcus* and *Chlamydomonas*. After inoculation the initial concentration of each species in the mixed culture was equal to that in the single-membered cultures. All flasks were grown side by side under the same light and temperature conditions for five days. At the end of that period the number of cells in each flask was determined. *Chlamydomonas* grew at approximately the same rate whether alone or in combination. *Haematococcus*

TABLE 1. *List of species of algae used in the present investigation*
The identifying strain numbers and, where possible, references to previously published work on the same strains are shown also.

Organism	Strain number*	References
Chlamydomonas reinhardi	I.U. 89	Sager and Granick (1953)
Chlamydomonas chlamydogama	I.U. 102	McVeigh and Brown (1954)
Haematococcus pluvialis	C.U. 34/1a	
Chlorella vulgaris	I.U. 263	Pratt and Fong (1940)
Scenedesmus quadricauda	I.U. 614	
Navicula pelliculosa	I.U. 673	Lewin (1955)
Anacystis nidulans	I.U. 625	Kratz and Meyers (1955)

* I.U. and C.U. = Indiana and Cambridge Universities.

TABLE 2. *Composition (g/liter) of the media used in the present investigation*
Stock cultures of all algae were maintained on solidified medium I.

	Medium					
	I	II	III	IV	V	VI
KNO_3	0.150	0.250	0.250	0.250		
NH_4NO_3						0.200
$(NH_2)_2CO$	0.050				0.150	
K_2HPO_4	0.100	0.100	0.100	0.100	0.100	0.100
$MgSO_4 \cdot 7H_2O$	0.100	0.100	0.100	0.100	0.100	0.100
Chelated iron*	0.005	0.005	0.005	0.005	0.005	0.005
Na_2SiO_3				0.100		
$NaC_2H_3O_2 \cdot 3H_2O$			1.000			
Proteose-peptone	5.000		5.000			
Dextrose	10.000					
Agar	20.000					
Soil extract:	all media contained 25 ml/liter					

* Supplied by Alrose Chemical Company, Providence, Rhode Island.

grew very well alone but would not survive for more than five days in combination with *Chlamydomonas*.

The results of such preliminary experiments involving five algae are shown Table 3. The relative growth of the algae in the ten possible two-membered combinations is shown as a percentage of that occurring in single-membered cultures grown under the same conditions. The figures are presented primarily as an example of the type of results obtainable. The percentages were not constant under different culture conditions, *e.g.* light, aeration, composition of the medium, and pH. The growth of the non-motile species was better on a mechanical shaker, whereas *Chlamydomonas* and *Haematococcus* consistently grew better in stationary cultures. Although the relative amounts of growth changed considerably, in general the same species always dominated the culture eventually.

TABLE 3. *The relative growth of five different algae grown in the 10 possible two-membered cultures shown at the left*

Growth of the algae in single-membered cultures under the same conditions is taken as 100%. All cultures were grown in Medium II.

Combinations	Rel. growth
Scenedesmus quadricauda	25
Chlorella vulgaris	100
Chlamydomonas reinhardi	25
Chlorella vulgaris	75
Chlamydomonas reinhardi	87
Scenedesmus quadricauda	12
Haematococcus pluvialis	20
Chlorella vulgaris	100
Haematococcus pluvialis	29
Scenedesmus quadricauda	38
Haematococcus pluvialis	0
Chlamydomonas reinhardi	100
Anacystis nidulans	100
Chlorella vulgaris	5
Anacystis nidulans	100
Scenedesmus quadricauda	10
Anacystis nidulans	100
Chlamydomonas reinhardi	0
Anacystis nidulans	100
Haematococcus pluvialis	0

From the preliminary experiments it was clear that both species in a two-membered culture seldom grew as well as when cultured separately. The cause of this general phenomenon is undoubtedly complex and probably varies with the combination of species. To understand the factors involved in even a single combination would require considerable study, and for this reason the *Chlamydomonas-Haematococcus* association was selected for more intensive study, primarily because these two species could be grown and maintained easily in the laboratory and distinguished readily under low magnification.

GROWTH OF *HAEMATOCOCCUS* AND *CHLAMYDOMONAS* IN MIXED CULTURES

When both *Chlamydomonas* (unless specifically stated otherwise, *C. reinhardi* was the species involved in the present investigation) and *Haematococcus* were inoculated into the same flask containing sterile Medium II and cultured under the usual conditions employed in this study they were found to be incompatible; the former invariably dominated the culture within a few days while the latter either formed resting spores or, more commonly, died. When the two were grown separately in the same medium and under the same light and temperature conditions, normal healthy cultures of each developed. After approximately the 8–10th day following inoculation *Haematococcus* began accumulating haematochrome and for all practical purposes stopped dividing. Cysts began to appear in the cultures several days later. The formation of haematochrome followed by encystment is a normal process in older *Haematococcus* cultures (Droop 1954, 1955). *Chlamydomonas* reached maximum cell density in about six days after inoculation. The *Chlamydomonas* cells then remained motile for at least another week although they were rather yellowish in color. The growth characteristics of a typical experiment are shown in Table 4. Similar experiments were conducted many times with essentially the same results; sometimes the death of *Haematococcus* in mixed culture

occurred a day sooner or later, but the alga never persisted beyond the sixth day.

The data in Table 4 may be somewhat misleading unless it is understood that *Haematococcus* cells are from five to ten times larger than *Chlamydomonas*. It was for this reason that cultures were routinely inoculated with more *Chlamydomonas* cells than *Haematococcus*. Within extremes the actual quantity of inoculum used was not too important except in determining the rate at which *Haematococcus* was eliminated from the mixed cultures. When *Haematococcus* was inoculated into a three to five day old culture of *Chlamydomonas* in Medium II the former species was killed within 24 hours. If on the other hand a three day old culture of *Haematococcus* was lightly inoculated with *Chlamydomonas*, it took from seven to ten days for *Chlamydomonas* to eliminate the other species and under these conditions *Haematococcus* was more likely to form cysts than to die.

It seemed improbable that the rapid inhibition and subsequent death of *Haematococcus* in the two-membered cultures could be accounted for by an unbalanced salt concentration in the medium. Neither *Chlamydomonas* nor *Haematococcus* could by the usual standards be considered fastidious species. Increasing or decreasing any or all of the components in Medium II by a factor of four had little effect on the growth rate of either alga. While a deficiency of nitrogen, the first element in the medium to be exhausted, might reduce the growth rate, it is hardly conceivable that a deficiency could develop in only six days to such an extent that it was able to cause the death of *Haematococcus*.

TABLE 4. *Changes in the cell density (thousand of cells/ml) and pH of unialgal and mixed cultures of* Haematococcus *and* Chlamydomonas *grown in Medium II*

	Days after inoculation			
	0	3	5	7
Haematococcus	3	37	134	210
pH	7.2	7.7	8.5	10.2
Haematococcus	3	11	dead	dead
Chlamydomonas	30	970	2,800	3,650
pH	7.2	8.6	10.1	10.5
Chlamydomonas	30	980	2,500	3,800
pH	7.2	8.5	10.0	10.3

In the preceding paragraphs the results of a typical experiment have been discussed. In addition, a number of experiments that were simply variations of the earlier one were conducted. The manner in which these variations and results differed from the preceeding will be summarized at this time. When the two species were cultured together under exactly the same conditions as above except that Medium II was replaced with Medium V, which contained urea rather than KNO_3, it took considerably longer for *Chlamydomonas* to eliminate *Haematococcus*. In Medium V the death of *Haematococcus* occurred on approximately the twelfth day. At that time there were about 10 million *Chlamydomonas* cells per ml.

The light intensity under which most experiments were conducted was approximately 200 foot-candles. When the two algae were grown together in Medium II under very low light intensity (10–25 ft-candles), *Haematococcus* persisted in the two-membered cultures for long periods of time. Cultures which were examined six weeks after inoculation contained 7.5 million *Chlamydomonas* and 5000 *Haematococcus* cells per ml. However at the same time there were 125,000 cells per ml in the parallel single-membered *Haematococcus* cultures. When cultures were grown under high light intensity (200 ft-candles) but illuminated for only 10 hours out of each 24, death of *Haematococcus* occurred at approximately the same time as in the cultures continuously illuminated at high light intensity.

Several minor experiments were conducted. In one the cultures were grown at temperatures of 31–34°C rather than at the usual 23°. The only difference noted was that *Haematococcus* was killed several hours sooner. When Medium II was prepared from unautoclaved Linsley Pond water, obviously contaminated with a number of other organisms, *Chlamydomonas* could still eliminate *Haematococcus* in approximately the same length of time. When mixed cultures of the two species were grown in Medium III, *Haematococcus* was elimi-

nated one day sooner than it had been from Medium II. Medium III contained peptone and acetate in addition to the usual inorganic salts present in Medium II. *Haematococcus* and *Chlamydomonas* were streaked in conventional configurations on agar plates (solidified Medium II) in an attempt to demonstrate the presence of a diffusable toxic substance. *Haematococcus* grew slowly on agar particularly under high light intensity, but there was no indication that it grew more slowly near the *Chlamydomonas* than at greater distances. When the two species were streaked close together, and after sufficient time they merged on the agar surface.

Because McVeigh and Brown (1954) were able to grow *Chlamydomonas chlamydogama* and *Haematococcus* together for two to three weeks with only minor mutual inhibition, a strain of *C. chlamydogama* was obtained and cultured alone and with *Haematococcus* in the usual fashion using Medium II. There was no indication that *C. chlamydogama* was able to inhibit the growth of *Haematococcus*, although the converse appeared to be true. The cell density of *C. chlamydogama* cultured with *Haematococcus* was only about one-half that in the single-membered culture.

EFFECT OF PH AND NITROGEN SOURCE

It gradually became apparent from the results of the preceding experiments that the death of *Haematococcus* in the two-membered cultures was closely associated with the interrelated problems of pH and nitrogen source. Nitrogen was supplied as KNO_3 in Medium II, and since nitrate ions are assimilated much faster than potassium ions (Krauss 1953) this probably accounted in large part for the rapid rise in pH from 7.2 to approximately 10.5. When urea was substituted for KNO_3 (Medium V) the death of *Haematococcus* occurred much later and at higher concentrations of *Chlamydomonas*. Davis and Dedrick (1953) have pointed out that in *Chlorella* cultures urea serves as an excellent nitrogen source without the wide pH fluctuations that result from the use of nitrate. This was also found to be true in the present investigation. The pH reached a maximum of only 8.3 twelve days after inoculation in Medium V. When the cultures were grown in Medium II under low light intensity (10–25 ft-candles), the pH rose to 7.5–8.0, and *Haematococcus* survived for six weeks in the two-membered cultures.

Chlamydomonas appeared to assimilate nitrogen considerably faster than *Haematococcus*. The pH of the two-membered cultures as well as that of the single-membered *Chlamydomonas* cultures was always higher than in the parallel single-membered *Haematococcus* cultures. This same point was also demonstrated by growing the two algae in Medium VI. When supplied with NH_4NO_3 as a nitrogen source, *Haematococcus* assimilated nitrate ions primarily, while *Chlamydomonas* took up ammonium ions. When a two-membered culture containing equal volumes of the two species was supplied with NH_4NO_3, the pH fell rapidly to 3.5, indicating a pronounced net uptake of ammonium nitrogen. *Chlamydomonas chlamydogama*, which did not adversely affect the growth of *Haematococcus*, is a much larger and more slowly growing species. The pH of two-membered cultures containing *C. chlamydogama* and *Haematococcus* was never higher than in the single-membered cultures of the latter.

In view of the apparent importance of pH to the survival of *Haematococcus* it was necessary to know the limits within which normal growth could occur. Fortunately this information was already available. As early as 1934 Elliot had been faced with the problem of determining the effect of pH on the normal growth of *Haematococcus*. From this study he concluded that pH values below 9.5 were favorable for the growth of motile cells. Dr. M. R. Droop has kindly supplied unpublished data which indicate that growth is normal and unaffected up to pH 9.5–10.0. The present writer has arrived at essentially the same conclusion. There is also general agreement that the lower value for normal growth is approximately 6.5.

From Table 4 it can be seen that the pH in Medium II reached 10.5 but that the growth of *Haematococcus* was clearly in-

hibited in mixed culture before the pH reached 9.5. In Medium V the pH was only 8.4 at the time *Haematococcus* was eliminated from the two-membered culture. Consequently it was concluded that although a strongly alkaline medium increased the toxicity of the factor responsible for the incompatibility of *Chlamydomonas* and *Haematococcus*, a high pH per se was not responsible for the death of the latter. This conclusion was supported by a study of the cell-free media from *Chlamydomonas* cultures.

EXPERIMENTS WITH CELL-FREE MEDIA

A number of experiments were conducted using cell-free *Chlamydomonas* medium, which was obtained from cultures grown either in 125-ml flasks or in aerated three-liter Fernbach flasks for three to six days.

TABLE 5. *Growth of* Haematococcus *after 3–5 days in fresh and conditioned*[1] *media treated in a variety of ways*

The results, expressed as a percentage of the growth occurring in the control, represent a condensation of a number of separate experiments.

Media and prior treatments	Initial pH	Relative cell density after 3–5 days
Freshly prepared Med. II (Control)	8.5	100
2 day old conditioned Med. II, unautoclaved	8.5	110–130
4–6 day old conditioned Med. II, unautoclaved	8.5	5–20
4–6 day old conditioned Med. II, unautoclaved	7.5	85–115
4–6 day old conditioned Med. II, autoclaved 10 min.	8.5	7–33
4–6 day old conditioned Med. II, autoclaved 2 hrs.	8.5	19–57
4–6 day old conditioned Med. II, autoclaved 2 hrs. at pH 5.5–6.0 and then adjusted to 8.5 and autoclaved an additional 10 min.	8.5	78–95
6 day old Med. II supplemented on 4th day with 0.2 g/liter of NH_4NO_3, autoclaved 10 min.	7.5	130
As preceding except autoclaved at pH 8.5	8.5	110

[1] "Conditioned" refers to the cell-free medium obtained from 2–6 day old cultures of *Chlamydomonas*.

At the end of that period the cells were removed by centrifugation, and the cell-free medium was either used immediately or frozen for future use. Additional salts, equal in composition and concentration to those initially present in Medium II, were added to the cell-free medium. The addition of only KNO_3 or urea gave equally satisfactory results. After the addition of salts, the pH was adjusted to the desired value with dilute HNO_3 or NaOH, the medium was autoclaved for ten minutes, and after cooling was inoculated with *Haematococcus*. The *Haematococcus* was left in the cell-free medium from 4–6 days, after which, providing any cells were still alive, the cell density was determined by direct count and compared with that in parallel cultures grown in fresh Medium II.

The results of a number of such experiments are summarized in Table 5. Since the results are a composite of a number of different experiments they are presented as percentages of the growth that occurred in the controls (fresh Medium II), which were subjected to the same light, temperature, and period of growth.

The results at first were conflicting, difficult to repeat, and highly reminiscent of the data presented by Lefevere *et al.* (1952). However, with greater attention to the age and pH of the culture medium, the relative toxicity was quite predictable. The effect of cell-free *Chlamydomonas* medium on *Haematococcus* was in part stimulatory as well as inhibitory. The net effect depended upon the age of the *Chlamydomonas* cultures from which the cell-free medium was obtained; from one and two day old cultures it was stimulating, while that from 4–6 day old cultures was decidedly toxic. The stimulatory effect of medium from one or two day old cultures probably was due to the conditioning of the medium. When *Haematococcus* was inoculated into fresh Medium II or V, there was a characteristic one to two day lag period. If, however, *Haematococcus* was inoculated into medium in which *Chlamydomonas* previously had been allowed to grow for one day or *Haematococcus* for two days and then removed, no lag occurred. Presumably conditioning en-

tailed the removal of small amounts of heavy metals from the medium, but the problem was not investigated further. Attention was paid primarily to the inhibitory effect.

In order to inhibit the growth of *Haematococcus*, it was not only necessary to obtain the medium from cultures that were at least 4 to 6 days old but the pH also had to be controlled. As shown in Table 5 cell-free medium that was decidedly toxic at pH 8.5 or above was at best only slightly toxic or occasionally even stimulating at pH 7.5 or below. Cell-free medium from 4–6 day old cultures grown in Medium II was considerably more toxic that that obtained from cultures grown either in Medium V or in Medium II under low light intensity. If cultures of *Chlamydomonas* were started in Medium II, grown for 3–4 days, and then supplemented with 0.2 g/L of NH_4NO_3 in sterile solution, the medium on the sixth day was not strongly alkaline but near neutrality, due to the preferential uptake of ammonium ions, and it was only slightly if at all toxic. Although adjusting the pH of 6 day old cell-free medium from 10.0 to 7.5 or below removed the toxicity, raising the pH of a neutral and non-toxic medium to 8.5 did not make it toxic. It was thus clear that the inhibitor was produced and active only in alkaline media.

HEAT STABILITY OF TOXIC PRINCIPLE

One reason for selecting the *Haematococcus-Chlamydomonas* association over the other nine possible two-membered combinations shown in Table 3 was the indication by preliminary experiments that the inhibiting factor in cell-free *Chlamydomonas* medium was at least partially heat stable. This was an obvious advantage from the viewpoint of pure culture technique. As shown in Table 5 the toxic cell-free medium could be autoclaved for at least two hours at 15 lb/in^2 without a loss of more that fifty per cent of the toxicity, although 10–15 minutes was generally sufficient to ensure sterility. Only distinctly alkaline media were heat stable when autoclaved; at pH 6.5–7.0 the inhibiting action of the medium was gradually, but almost entirely, dissipated over a two-hour period.

During preliminary experiments on heat stability the cell-free medium was sometimes autoclaved, and at other times refluxed or even simply boiled for the desired length of time. At the suggestion of Mr. J. Shapiro, careful attention was paid to the vapor escaping from the heated medium. It was soon apparent that the condensate from a boiling acidified *Chlamydomonas* medium inhibited the growth of *Haematococcus* in the same manner as the centrifuged cell-free medium. If the medium was boiled gently to prevent excessive frothing and the steam carried through a water-cooled condensor column packed with glass wool or beads, most of the toxic substance collected on the column and packing as a yellowish-white film or droplets. Only a trace, which was difficult to see without magnification, was obtained from cell-free medium. Considerably larger amounts were obtained from the cells. When one liter of 4–6 day old *Chlamydomonas* culture was harvested and, without any further centrifugation, the cells and medium heated to boiling, then acidified, and slowly distilled over through a water cooled condenser, several mg of the toxic material could be obtained. This material was soluble in a number of organic solvents. It was insoluble in water but dissolved slowly in a basic aqueous solution. It was decidedly toxic: *Haematococcus* cells were completely killed within 24 hours after inoculation.

DIFFERENCES IN TOXICITY BETWEEN CULTURES

Upon finding that the inhibitor was many times more concentrated in the cells than in the cell-free medium the earlier results became more meaningful. As pointed out previously, cultures grown under high light intensities were more toxic than those reared under weaker light. Nitrate supplied (Medium II) cultures were more toxic than those containing urea (Medium V). The principal differences between *Chlamydomonas* cultures grown in different media or under different light conditions appeared on the 4th or 5th day after inoculation.

Although most of the cells remained motile in both media for several weeks, a proportionately higher number of cells died in Medium II than in V, presumably because of the higher pH attained in the former cultures supplied with nitrate. *Chlamydomonas* grew very slowly under low light intensity, but nearly all cells remained dark green and motile for long periods. It thus seemed probable that Medium II was more toxic than V simply because more *Chlamydomonas* cells had died, thereby allowing more of the inhibitor to be liberated into the medium from the cells. As a test of this hypothesis, a number of flasks each containing 50 ml of sterile Medium II adjusted to pH 8.2 were inoculated with sufficient cells from a pure culture of *Chlamydomonas* to give initial concentrations of 100, 50, 10, 5, 1, and 0 thousands of cells per ml. The inoculum was taken from a culture grown under low light intensity containing 5 million cells per ml. The cell-free medium from this culture was assayed and found to be non-toxic. Immediately after inoculation the flasks and contents were frozen solidly, held for 36 hours, and then thawed. All *Chlamydomonas* cells had been killed. At that time the flasks containing the dead *Chlamydomonas* cells were inoculated aseptically with *Haematococcus*. On the third and fifth days following inoculation the cell density of *Haematococcus* in the flasks was determined. The results, shown in Figure 1, indicate that large quantities of *Haematococcus* inhibiting material can be liberated following the death of only a small percentage of the cells normally present in a 6 day old culture.

Attempts to extract the inhibitor from the cell-free medium by the use of organic solvents were by and large unsuccessful due to the rapid formation of stable emulsions whenever the medium and solvents were shaken. After centrifugation the medium always contained a small amount of mucilaginous debris, including cell walls and broken flagella, which was extremely difficult to remove completely. This debris was probably responsible for the emulsions. It was impossible to remove the mucilaginous material by ultrafiltration. Filters clogged completely after the passage of only a few milliliters of the cell-free medium.

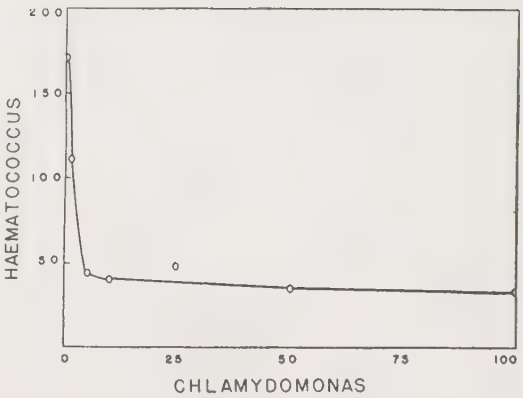

FIG. 1. Effect of killed *Chlamydomonas* cells on the subsequent growth of *Haematococcus* in the same medium. The number of *Chlamydomonas* cells (thousands of cells/ml) that were killed in the cultures is shown on the abscissa. On the ordinate is shown the number of *Haematococcus* cells (thousands of cells/ml) that developed after five days.

For reasons that will be discussed more fully in a later section, it seemed most probable that the death of *Haematococcus* in two-membered cultures was due in large part to long-chain fatty acids that were liberated into the medium from the small percentage of dead *Chlamydomonas* cells always present in older cultures. The toxicity of the fatty acid was also enhanced by the higher pH associated with nitrate assimilation. At this point in the study there were two possible courses that seemed profitable to follow: (1) the quantitative estimation and identification of the fatty acids present in *Chlamydomonas* cells, followed by proof that a sufficient quantity of these acids was present in the medium to account for the death of *Haematococcus*, or (2) a study of the effects of long-chain fatty acids on freshwater algae, with particular reference to *Chlamydomonas* and *Haematococcus*, in order to determine the relative toxicities of the different acids against the algae under the conditions of the present experiment. The second course was selected primarily because of personal choice. Obviously both lines of research will have to be fol-

lowed ultimately in order to achieve the overall objectives of the study.

EFFECT OF KNOWN FATTY ACIDS ON SIX ALGAE

Considerable information has accumulated over the past two decades concerning the effects of long-chain fatty acids on different bacteria and fungi (see reviews by Kodicek 1949, and Nieman 1954), but apparently little is yet known regarding the effects of these acids on even the more commonly studied algae. To this end a study was undertaken to ascertain the effects of eight C_{9-18} saturated and unsaturated straight-chain fatty acids on the growth of the following algae: *Chlamydomonas*, *Haematococcus*, *Chlorella*, *Scenedesmus*, *Navicula*, and *Anacystis*.

Sodium soaps were prepared from commercially available (Eastman and Fisher) reagent and purified grades of the eight fatty acids. The soaps were added in a graded series of concentrations to flasks of Medium IV adjusted to pH 8.2. After autoclaving, the flasks were inoculated with the different algae and placed in the light room. The non-motile species were grown on a mechanical shaker and the two flagellates in stationary cultures. All of the algae grew normally in Medium IV, but some of the species grew more rapidly than others. Since the primary object of the study was to determine the concentrations of the different fatty acids required to inhibit the growth of the different algae by approximately fifty per cent, it was necessary to grow some of the species for longer periods before comparing the growth attained in the fatty acid containing flasks with that in the acid-free controls. *Anacystis*, *Chlorella*, and *Chlamydomonas* grew very rapidly, and results were apparent in two or three days, *Scenedesmus* and *Haematococcus* were slower growing, and *Navicula*, which had a pronounced lag period, required 6–8 days.

Tests of the different acids, algae, and concentrations were carried out in duplicate. As a result it was not feasible to conduct the entire experiment at one time due to the large number of flasks required. Concentrations of acid of 100, 50, and 25 mg per liter were first tested. Several of the algae were not inhibited by concentrations as high as 100 mg per liter. It seemed improbable that concentrations higher than this would ever be encountered in natural water. Consequently the results in these cases were indicated simply as >100 and were not investigated further. Those species that were inhibited by 50 mg per liter were then tested at lower and lower concentrations until the fifty per cent inhibition level was reached.

The results are shown in Table 6. Of the algae tested *Chlorella* and *Scenedesmus* were the least sensitive to the fatty acids and *Haematococcus* was the most. Presumably even more sensitive species could be found if a wider range of algae were tested. Mycologists have also observed that different fungi exhibit marked variations in their reaction to fatty acids. *Penicillium chrysogenum* was able to oxidize palmitate and stearate at concentrations as high as 10 g per liter (Rolinson 1954), while *Neurospora crassa* was inhibited by concentrations of 50 mg per liter and lower (Lein and Lein 1950). In general palmitic appeared to be the most toxic of the saturated acids although only slightly more so than were lauric, myristic, and stearic. As might be expected oleic was less toxic than the more

TABLE 6. *The minimum concentrations (mg/liter) of six saturated and two unsaturated fatty acids required to inhibit the growth of six algae by approximately fifty per cent when grown in Medium IV at pH 8.2*

Organism	Fatty acid							
	Nonanoic	Decanoic	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic
Chlorella vulgaris	>100	>100	>100	>100	25	50	100	50
Scenedesmus quadricauda	100	>100	>100	>100	>100	>100	>100	>100
Chlamydomonas reinhardi	>100	>100	25	4	3	5	5	5
Haematococcus pluvialis	5	5	5	5	3	5	3	2
Anacystis nidulans	100	>100	10	4	5	—	4	3
Navicula pelliculosa	50	50	5	5	3	5	4	3

unsaturated linoleic. With the exception of nonanoic and decanoic, the range of concentrations between complete inhibition and no inhibition was very narrow. For example, 2 mg per liter of oleic acid had no appreciable effect on the growth of *Haematococcus* while 5 mg caused nearly complete inhibition.

Doubtlessly the effects of pH, solubility, and a number of other factors influenced the relative toxicity of the different acids to the different algae. In a preliminary experiment of this type there was not time to consider all these possible ramifications. However, one short experiment concerning the effects of pH on the toxicity of palmitic and oleic acids to *Chlamydomonas* and oleic to *Haematococcus* was conducted. Different concentration of these fatty acids were added to flasks containing Medium V and the pH adjusted to the desired value. The flasks containing the medium plus the fatty acids were then autoclaved, and inoculated with *Chlamydomonas* and *Haematococcus*. After 4 or 5 days the cell density in each flask was determined. From the results, which are shown in Figures 2 and 3, it can be seen that the inhibiting action is considerably greater in alkaline than in neutral solution. Although many cells were killed

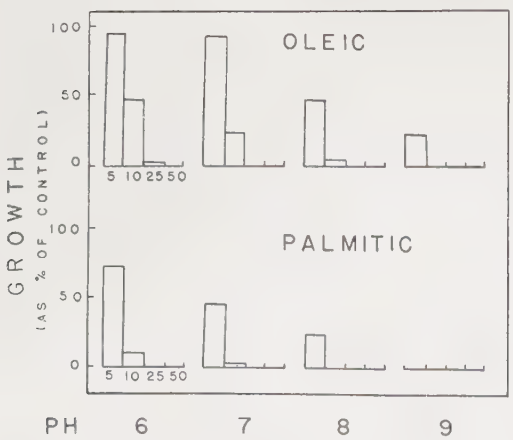

Fig. 2. Effect of palmitic and oleic acids on the growth of *Chlamydomonas* in Medium V. Concentrations of 5, 10, 25, and 50 mg/L of each acid were tested as the sodium salts at pH 6, 7, 8, and 9. Cell density, determined five days after inoculation, is shown on the ordinate as a percentage of that occurring in the control (acid free) at the same pH.

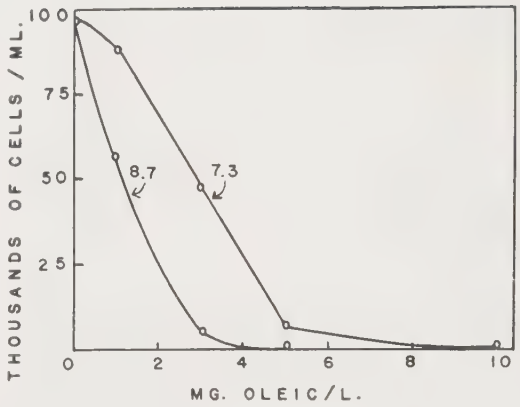

Fig. 3. Effect of oleic acid on the growth of *Haematococcus* at pH 7.3 and 8.7 in Medium V. Cell density, determined four days after inoculation, is shown on the ordinate.

when inoculated into the higher concentrations of fatty acid, some always survived, and after a period of from one to three weeks slowly began to divide, giving rise to cultures of normal appearance. Whether the eventual growth was due to the decomposition of the fatty acid or the ability of the algae to overcome the toxicity was not clear.

Wynne and Foster (1950) were able to counteract the toxic effects of unsaturated fatty acids on bacteria by the addition of either cholesterol or lecithin. In the present study several unsuccessful attempts were made to overcome the effects of oleic and linoleic as well as palmitic acid on *Chlamydomonas* and *Haematococcus* with cholesterol and lecithin. The concentrations of cholesterol and lecithin used were not toxic to either alga when tested alone. They did not increase the toxicity of the fatty acids nor did they decrease it.

The six algae were streaked side by side on several petri dishes of solidified medium, each containing from 1 to 10 mg per liter of a different fatty acid. All six algae grew on the agar at all concentrations tested. Subsequently it was found that concentrations 10–100 times greater were required in agar than in liquid to give equal inhibition. Lewin (1952, unpublished) tested several long-chain fatty acids in agar in the presence of glycerol and Tween 80 against the same strain of *Navicula* used in the present study

and found the lowest inhibiting concentrations of oleic and lauric acid to be 0.4 and 1.0 g per liter, respectively.

DISCUSSION

In the present investigation it has been shown that *Haematococcus* and *Chlamydomonas* are incompatible when grown together. The death of *Haematococcus* results from a toxic substance present in the medium. The toxic substance is present in highest concentration within the *Chlamydomonas* cells, but is released into the surrounding medium upon the death of the cells. Early in the investigation it was noted that cultures grown in some media became toxic sooner than others. Such differences can be explained in terms of the percentage of cells that had died in the cultures. When *Chlamydomonas* cultures were grown under high light intensities in nitrate-containing media, growth was very rapid for short periods of time, but at the end of this period many of the cells died. Under lower light intensities and/or in a urea medium growth was slower but extended over longer periods and fewer cells died in the older cultures. The inhibitor was considerably more effective in alkaline than in neutral or acidic media, and the final pH was considerably higher in the cultures grown under high light intensity and supplied with nitrate.

There have been five previous studies on algal inhibition that are sufficiently detailed to allow a comparison of the results with one another and with the present investigation: Pratt (1948), v. Denffer (1949), Lefevre *et al.* (1952), Harder and Oppermann (1953), and Rice (1954). The work of Spoehr *et al.* (1949) was closely associated with that of Pratt and should be included in a consideration of the latter's work. When the results of all these studies are compared, a number of common experimental procedures and observations are noted.

Most workers have employed an unbuffered or only weakly buffered medium. Nitrogen in every case was supplied as either sodium, potassium, or calcium nitrate, or as a mixture of the three. As a result of the preferential assimilation of nitrate ions an increase in pH occurred. In most cases the pH rose from near neutrality to somewhere between 9 and 10. While Harder and Oppermann did not report the maximum pH attained in their cultures, the composition of the medium employed would indicate a maximum of 9.0 or greater.

The light intensities employed in the different studies varied from less than 200 to nearly 1000 ft-candles. Most workers have observed that higher concentrations of the inhibitor are associated with increased light intensity. Spoehr *et al.* grew *Chlorella* in a greenhouse, and Lefevre and coworkers grew a number of different algae in a north window of the laboratory. Both noted that the cultures became toxic to other species much sooner in late spring and early summer than during the winter months. Similar results were obtained by Pratt and v. Denffer using different intensities of artificial illumination. This has led some investigators to conclude that under low light intensity less inhibitor accumulates either because less is synthesized or more is utilized than under higher light intensity. In the opinion of the present writer a somewhat different explanation is suggested by the data.

Most workers have agreed that the inhibiting substances are produced most rapidly under conditions favorable to maximum growth. In microbiology there are two conceptions of maximum growth: the maximum rate of growth and the maximum total yield. Presumably the former concept is the one to which most workers have alluded. But conditions which result in a maximum growth rate may also result in a larger percentage of dead cells than would be present in a slower growing population. From a careful consideration of previous studies it seems quite probable that most investigators have worked with cell-free media obtained from senescent or otherwise unhealthy cultures. In some cases the algal cultures were allowed to grow for long periods of time, while in others the unhealthy condition of the cultures was brought about by high light intensity and/or increased pH of the medium, but in either case the cultures contained a sizeable percentage of dead cells.

Pratt found an algal inhibitor, chlorellin, present in older *Chlorella* cultures. Chlorellin began to accumulate slowly in the medium two to three weeks after inoculation. It was not found in the rapidly dividing cultures. However, it was present on the first and second days after inoculation while the cells were still in the lag phase, in concentrations very nearly as great as at the end of the second week. This initial concentration diminished rapidly as the cells began to divide. When algal cells are inoculated into fresh medium, a small percentage of the cells usually fail to survive the transfer. As shown earlier in the present investigation (see Fig. 1) the death of only ten per cent of the *Chlamydomonas* inoculum released sufficient toxin to inhibit the growth of *Haematococcus* by nearly fifty per cent. The initial concentration of chlorellin observed by Pratt may have resulted from the death of a small percentage of the inoculum.

Myers, Phillips, and Graham (1951) using a different strain of *Chlorella* and somewhat different culture conditions found autoinhibitors to be of little importance in the limitation of growth.

Nitzschia palea was grown in an inorganic medium by von Denffer who observed that the cells stopped dividing on approximately the fifth or sixth day after inoculation and before any of the essential elements in the medium were exhausted. In these cultures the pH of the medium rose from 8.7 to a maximum of 10.2 on the sixth day. At approximately this same time the cells stopped dividing, although the fat content of the diatoms continued to increase for at least another week.

The investigations of Lefevre, Jakob, and Nisbet (1952) were concerned primarily with the growth of *Pandorina* and *Scenedesmus*, although a number of other species were mentioned in less detail. Cultures ranging in age from six weeks to seven months were used. In these investigations little is said concerning the condition of the cells in the cultures, but it seems probable that cultures of that age would have contained large numbers of senescent or dead cells. In at least one case (growth curve on page 72) it is clear that the cultures already had begun to decline at the time the presence of the inhibitor was demonstrated. It is doubtful whether the "conditioned" medium used in these experiments was actually cell free. In at least some of the cases reported the cultures were neither filtered nor centrifuged before autoclaving, but simply separated by decantation. From the limited data available it would appear that the pH of most cultures rose from 7.0 to somewhere between 8.0 and 9.0. It is rather probable that almost any algal culture regardless of the age, medium, or culture conditions would be found toxic to some extent if it were autoclaved at pH 8.5 before all the cells were removed.

Harder and Oppermann (1953) obtained an antibacterial substance from old cultures of *Protosiphon* and *Stichococcus*. The algae were grown for three to five weeks under continuous illumination from a water-cooled 1000-watt bulb before the cell-free medium was tested. While there is no doubt in this case that the antibiotic was present in the cell-free medium, it is not clear whether it was secreted by living cells or was simply a decomposition product of dead cells. After an interval of three weeks under such high light intensity, it seems improbable that a majority of the cells were still alive.

The most carefully conducted study of algal inhibition yet reported was that by Rice, which concerned the antagonistic effects of *Chlorella* and *Nitzschia* on one another when grown together. The maximum age of Rice's cultures was only seven days. Very complete data regarding cell density, pH, and phosphorous concentrations were kept. Even in these cultures the pH rose from 7.2 to a maximum of 9.0 by the third or fourth day following inoculation, and it was only after the fourth day that marked inhibition in the mixed cultures was noted. While the cultures used in this study were in better physiological condition than in any other investigation, it is also worth noting that the inhibition observed by Rice was considerably less than that reported by Lefevre or shown in the present investigation.

In the present investigation the algae

were grown for six days or less. Within that period a marked increase in the pH occurred in the *Chlamydomonas* cultures. By the fifth day following inoculation the *Chlamydomonas* cultures grown under high light intensity in Medium II had reached maximum cell density. Although a majority of the cells remained motile for at least another week, microscopic examination on the fifth and sixth days disclosed a large number of lysed cells.

A number of investigators have demonstrated the presence of antibiotic substances in algal cultures, but conclusive proof of whether this inhibition is due to secretions of healthy cells or the decomposition products of dead cells is still lacking. It has yet to be shown that an inhibiting substance can be obtained from a strictly healthy culture of algae growing in a neutral solution. In either case Akehurst's original hypothesis would be, in general, correct. Wide pH fluctuations in ponds and shallow pools can and do occur, and a majority of the algal cells produced in such bodies of water are not immediately consummed but die and liberate the cell contents into the surrounding water. In the case of blooms, enough of the inhibiting substance might be liberated to account for the changes that occur in the algal flora.

Although there are some indications of the chemical composition of the algal inhibitors, definite information on the subject is not yet available. Pratt did not identify chlorellin, the growth inhibiting substance present in *Chlorella* cultures. Spoehr, working on an antiobiotic substance which he also called chlorellin, came to the conclusion that the antibacterial activity of the substance was due in large part to a mixture of photooxidized unsaturated fatty acids. The mixture of fatty acids used by Spoehr for identification purposes was obtained from *Chlorella* cells. The chlorellin with which Pratt worked was obtained from the cell-free medium of *Chlorella* cultures. Presumably the substances are at least similar but proof of this point is needed.

Harder and Oppermann also came to the conclusion that the antibacterial activity of concentrated cell-free medium from aged cultures of *Protosiphon* and *Stichococcus* was due to unsaturated fatty acids. However this conclusion was based upon little more than the fact that the medium had a yellowish-orange color and a rancid odor. A repetition of this work using more adequate identification procedures would be most helpful.

Although the inhibiting substance present in *Chlamydomonas* cells and toxic to *Haematococcus* has fat-like properties in general, it has not yet been identified. The inhibitory effects of the *Chlamydomonas* toxin and of the known fatty acids are quite similar. From the concentrations of fatty acids required to cause inhibition and the amounts that might be expected in the *Chlamydomonas* cells the explanation is quite possible. It can be concluded from the present study that concentrations of 2–5 mg per liter of the long-chain fatty acids would be required to inhibit the growth of *Haematococcus*. Investigations by Collyer and Fogg (1955) have shown that the fatty-acid content of most green algae is between 10 and 30 per cent of the dry weight of the cells. Seventy-five per cent of these acids are probably unsaturated; the remainder is a mixture consisting principally of palmitic, stearic, and myristic acids (Milner 1948, and Eckey 1954). If the not unreasonable assumption is made that the majority of the unsaturated acids have a carbon chain length of 16 and an average of two double bonds per molecule, simple calculations disclose that, if free, the unsaturated fatty acids from only 5–10 per cent of the total amount of *Chlamydomonas* cells could account for the observed inhibition of *Haematococcus*. The saturated acids although present in lesser amounts are almost equally toxic. The increased pH generally associated with older cultures would intensify considerably the toxicity of the fatty acids, as well as aid in the liberation of the salts of the free fatty acids by mild saponification of the triglycerides present in the cells.

Several cases of antagonistic and/or stimulating interaction between different organisms have been ascribed to the effects of saturated and unsaturated fatty acids. Pollock (1949) has reported on the inter-

esting association of the bacteria *Haemophilus pertussis* and *Corynebacterium* "Q". When cultured separately these two organisms developed very poorly. *Haemophilus* was soon autoinhibited by toxic concentrations of unsaturated fatty acids, while *Corynebacterium* was unable to grow unless supplied with these same acids. When grown together in a mixed culture the development of both species was much better due to what might be called a "Jack Spratlike" relationship.

Recently James and Wheatly (1956) have been able to show conclusively by the use of gas-liquid chromatographic procedures that, as long suspected, the inhibiting effects of human sebum on several bacteria are due to a mixture of fatty acids.

At the present time it seems probable that the observed inhibition of *Haematococcus* by *Chlamydomonas*, as well as many of the results of the five previous investigations, may be explained in terms of the different concentrations of various fatty acids required to inhibit the different algae. Nevertheless, promising as this explanation appears to be at the present time, it must be admitted that final proof of the chemical nature of the inhibiting substance is still lacking. Rapid advances in chromatographic procedures for the identification of trace amounts of long-chain fatty acids should provide a definite answer to the interesting question of algal inhibitors in the near future.

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Short-Term Variations in Phytoplankton Chlorophyll and Their Significance[1]

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ABSTRACT

Diurnal fluctuations were observed in the chlorophyll *a* content of natural phytoplankton populations, with the highest concentrations during the morning and afternoon, low values at midday and at night. This periodicity is similar to the daily rhythm of photosynthesis with which it appears closely related. The rate of synthesis of chlorophyll at optimal light intensity is dependent upon its previous rate of synthesis and concentration in the organisms. All of these factors strongly influence the capacity for photosynthesis at optimal light intensity and are probably the basis for the photosynthetic daily periodicity reported elsewhere.

INTRODUCTION

Recent experiments by Doty and Oguri (1957) have indicated a daily periodicity in the photosynthetic capacity of planktonic algae under constant illumination. Samples taken from the same population showed a seven-fold difference in their ability to assimilate C^{14} in an illuminated incubator, between the minimum at midnight and the maximum at 1000 hrs.

Marked diurnal variations in the chlorophyll content of surface populations of phytoplankton have been noted on numerous occasions by the senior author in Woods Hole (Mass.), East Sound, (Wash.) and elsewhere (Yentsch, in preparation). It is the purpose of this paper to investigate the possible relationship between the diurnal *in situ* fluctuations in the chlorophyll *a* content of phytoplankton and their photosynthetic capacity when exposed to a constant illumination.

METHODS AND RESULTS

Surface water from Woods Hole Harbor was enclosed in two carboys which were suspended at the water surface for a period of 24 hours. At four-hour intervals two one-liter samples were withdrawn, the first of which was used for the immediate determination of chlorophyll, while the second was placed in a glass container and exposed to 2000 foot candles of artificial illumination in a water-cooled incubator for four hours after which its chlorophyll content was measured. The samples were millipore filtered, their pigments extracted according to Creitz and Richards (1955), and chlorophyll *a* determined quantitatively by the spectrophotometric method of Richards with Thompson (1952).

At the same time the chlorophyll samples were taken, an additional 150 ml sample was withdrawn, enclosed in a glass-stoppered bottle, inoculated with approximately 5 μ curies of $C^{14}O_3^=$, and placed in the incubator for 15 minutes. After this exposure, the samples were millipore filtered, the filters dried, and their radioactivity, in counts per minute, taken as an index of relative photosynthesis. The experiment ran from 1200 on July 18 to 1200 on July 19, 1956, under completely clear skies. Solar radiation, measured with an Epply pyrheliometer, reached a maximum of approximately 1.5 g-cal/cm^2/min. at noon of each day (Fig. 1A).

A diurnal variation was observed in the photosynthesis at 2000 foot candles of samples taken from the *in situ* carboys (Fig. 1B). This was similar in its periodicity to that reported by Doty and Oguri, with a maximum at 0800 and a minimum at 2400, but the magnitude of the variation was less, with only a two-fold range.

Chlorophyll *a* samples taken directly

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from the carboy showed a similar diurnal variation. Highest values were observed at 0800 while the lowest were at 1600. Chlorophyll *a* values increased in all samples after a four-hour exposure to 2000 f.c. The maximum rate of increase was at 0800 when the chlorophyll content was at a maximum, while the lowest rate occurred at 0400, again duplicating the daily photosynthetic pattern. *In situ* rates of pigment increase were highest during the hours of low or no illumination. Low rates of increase at 2000 f.c. and high rates of *in situ* decrease occurred during the hours of intense illumination.

In situ fluctuations in the amounts of chlorophyll are in excess of what could be expected by cell division or death alone.

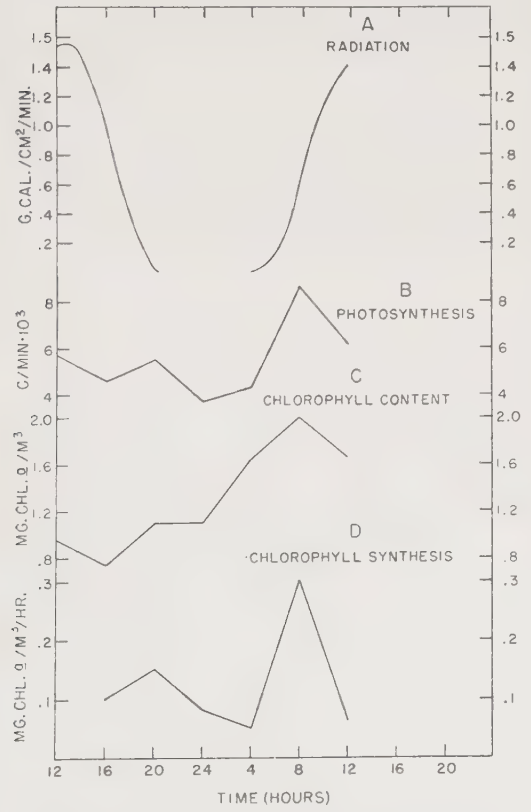

FIG. 1. (A) *In situ* solar radiation, (B) photosynthesis at 2000 foot candles as measured by 15-minute C^{14} experiments, (C) *in situ* chlorophyll *a* content, (D) chlorophyll *a* synthesis at 2000 foot candles (each point represents synthesis during previous four hours).

TABLE 1. *Rates of pigment change throughout twenty-four hours under different conditions of illumination*

Sampling time	Chlorophyll *a* change during the following four hrs in mg/m³/hr.	
	In situ	At 2000 f.c.
1200	−.21	+.10
1600	+.32	+.15
2000	+.02	+.08
2400	+.54	+.05
0400	+.35	+.30
0800	−.40	+.06

A decline of the chlorophyll within the cell may result from photo-oxidation of the pigmented substrates when photosynthesis is inhibited, (see Rabinowitch, Vol. 1, pp. 355–571). When light conditions are optimal for photosynthesis pigment formation usually proceeds rapidly. Thus, the alternation of low and high light intensities throughout the day results in a diurnal fluctuation in the amount of chlorophyll in surface plankton algae.

During the experiment the rate of chlorophyll synthesis under constant illumination varied considerably (Table 1). From these data it would appear as though the synthesis of the chlorophyll-protein complex was dependent upon the level of the respiratory carbon in the cell prior to the four-hour exposure to 2000 f.c. In any event, the data illustrate the difficulty encountered in comparing an instantaneous measurement of chlorophyll with a long-term measurement of photosynthesis.

These experiments illustrate the interrelationship between chlorophyll content, chlorophyll synthesis, and photosynthesis of plankton algae exposed to constant illumination. In this relationship it is difficult to distinguish between cause and effect. But it seems clear that the daily periodicity in the photosynthetic capacity of the phytoplankton, as reported by Doty and Oguri and confirmed here, may be at least partially explained on the basis of the previous history of the organisms in terms of their chlorophyll content, which in turn is light dependent and varies diurnally with the intensity of solar radiation.

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The Chemical Composition of Some Natural Waters in the Cairn Gorm-Strath Spey District of Scotland[1]

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ABSTRACT

Some chemical properties of natural waters in the Cairn Gorm-Strath Spey area have been investigated. Differences of ionic concentrations in twenty-four waters analyzed for pH, Na, K, Ca, Mg, HCO_3, Cl, SO_4, NO_3, PO_4 and SiO_2 are interpreted in terms of variation in geology, topography, local climate and vegetation.

INTRODUCTION

In recent years the ecology of the Cairn Gorm mountains has been studied in some detail by Cambridge botanists (Watt and Jones (1948), and the establishment of a large nature reserve in the area will no doubt stimulate interest in its flora and fauna. The aim of the present paper is twofold: first, to contribute some background data on the chemistry of the waters, with especial reference to dissolved constituents in the waters of upland and lowland lakes, and second, to examine the influence of environmental factors upon chemical composition.

DESCRIPTION OF THE AREA

The general area of this survey, covered by sheet 43 of the Ordnance Survey inch-to-the-mile map of Scotland, is well described in the National Forest Park Guide *Glen More*, edited by Walton (1949). The main mountain mass making up the Cairn Gorm plateau, about 1100–1200 m high, is of granite intruded into the metamorphic Moine Series of schists and gneiss, which in the Spey valley is covered by a thick mantle of glacial deposits composed of varying mixtures of sand, gravel, and clay. Many of the lakes owe their existence to damming by moraines, while Loch Morlich is a large kettle-hole left after the melting of a huge ice-block in the valley. The detailed geology of the district has been discussed by Hinxman and Anderson (1915) and their collaborators.

Rainfall in the valley is approximately 75 cm per annum, and mean annual temperature about 7.5°C, with the three summer months averaging about 13°C. In the uplands conditions are of course much more severe, but unfortunately no data are available. However, it may be noted that at the end of July, in the very warm summer of 1955 when the water samples were collected, occasional snow patches were still to be found in protected places near the summits of the hills. According to Watt and Jones (1948) the hills may be covered by snow for short periods in September, and snow lies generally from October to May or June on the upland plateau.

The vegetation of the valley glacial deposits, which has been described briefly by Tansley (1939), is largely open pine forest and heath, which may extend to above 450 m in many places, and occasionally above 600 m. Above these levels is found a more montane group of heath and moorland communities, whose cover gradually thins out above about 750 m. The upland areas have been described recently by Watt and Jones (1948), Metcalfe (1950) and Burges (1951).

THE SOILS

The soils of the region are commonly acid and base-deficient at the surface, as has been

[1] The water analyses for nitrate, phosphate, and silica were kindly made by Mr. J. Heron, and the total nitrogen analyses of the soils by my wife, who also assisted in their collection. Financial assistance from the Central Research Fund of London University during the soil investigations in 1949 is also gratefully acknowledged.

TABLE 1. *Some chemical properties of upland soils on Cairn Gorm and lowland soils near Loch Morlich*

A. Characteristics of some shallow upland soils.

pH	Water content	Ignition loss	Total nitrogen	Exchangeable cations*			Base saturation	
				Total bases	Hydrogen ions	Exchange capacity		
glass electrode	per cent dry weight			m.equiv./100 g dry weight			per cent	
4.5	10	4	0.16	12	3	15	80	Beneath patches of alpine vegetation at about 1150 m
4.3	25	5	0.17	9	5	14	64	ditto
4.1	15	6	0.22	7	5	12	58	ditto
4.0	20	6	0.24	5	5	10	50	ditto
3.9	10	6	0.19	5	5	10	50	ditto
3.8	165	48	1.38	17	34	51	33	*Nardus stricta—Trichophorum caespitosum* flush at about 750 m
3.5	155	53	1.49	9	42	51	18	Beneath patches of alpine vegetation at about 1150 m
3.3	165	72	1.25	11	63	74	15	*Trichophorum caespitosum—Calluna vulgaris* moorland at about 600 m

B. Acidity of humus layers in open pine-heath.

Sample	pH
1. *Mor* humus layer (30 cm thick) on sandy podzol with grey A_2 horizon (8 cm thick)	3.2
2. *Mor* humus layer (10 cm thick) on sandy podzol with grey A_2 horizon (20 cm thick)	3.1
3.–5. *Mor* humus layers (5–10 cm thick) over shallow greyish A_2 horizons, on top of fossil podzols buried by wind-blown sand	3.3 3.0 2.9

C. Characteristics of a lowland podzol profile in pine-heath

Horizon	Thickness of horizon	Depth of sample	pH	Water content	Ignition loss	Total nitrogen	Exchangeable cations*			Base saturation
							Total bases	Hydrogen ions	Exchange capacity	
	cm		glass electrode	per cent dry weight			m. equiv./100 g dry weight			per cent
A_0 (F)	7	3	3.3	350	84	1.76	15	104	119	14
(H)	8	11	3.0	335	92	1.42	7	106	113	6
A_2	8	19	3.9	15	1	0.05	1	2	3	33
B_1	10	28	4.4	35	11	0.23	19	17	36	53
B_2 iron pan	ca. 0.2	33	5.1	25	12	0.19	24	9	33	73
B_2	>22	37	5.7	10	3	0.07	16	1	17	94
B_2	>22	50	6.0	5	1	0.09	9	1	10	90

* By the method of Brown (1943)

established by investigation of a series of upland soils on Cairn Gorm and a representative lowland podzol profile near Loch Morlich in 1949, followed by measurement of the acidity of five surface *mor* humus layers in open pine-heath near Loch Morlich in 1952. The results of the soil analyses are shown in Table 1.

The upland soils are all strongly acid (pH 3.3–4.5), and while the peaty organic samples exhibit the lowest pH values, it is remarkable that even the least organic soil yields a pH of 4.5. In this connexion it should be pointed out that all samples were collected beneath patches of vegetation, and no bare soils were examined. In the lowlands the highly organic surface *mor* layers are even more strongly acid than the peaty upland samples, with a pH range of only 2.9–3.3. It seems probable that sulphuric acid, produced by oxidation of organic sulphur compounds, is responsible for these

very high acidities (cf. Gorham 1956b). From Table 1 it is also apparent that the base saturation of the soil adsorptive complex, determined chiefly by the amount of exchangeable hydrogen ions present, is related rather closely to pH and humus content.

The soil profile was chosen as fairly representative for the area, and had a plant cover of open pine-heath, with *Calluna vulgaris*, *Vaccinium myrtillus*, *V. vitis-idaea* and *Hylocomium splendens* abundant among the scattered Scots pines. It is developed in a stony sand near Loch Morlich, and probably overlies laminated fluvioglacial sands as in several road-cut exposures in the vicinity. The profile is typical of sandy podzol soils, with a thick and strongly acid humus layer above a heavily leached and base-deficient grey A_2 sand horizon. Beneath in the very stony and compacted sand of the B accumulation zone there is a marked increase of organic matter, exchangeable hydrogen ions, and exchangeable bases, while pH and base saturation rise considerably. An extremely impenetrable cemented hardpan of a few mm thickness, observed at 33 cm depth, marks the level of maximum iron precipitation.

THE WATERS

This district exhibits a great variety of lake types. In size the present series ranges from Loch Einich, about 3 km long and 0.8 km wide, down to very small lochans of less than 100 m across; and in altitude from just under 1000 m down to about 200 m above sea level. In the warm weather of July 1955 the surface waters of even the highest lochans ranged in afternoon temperature from about 20 to 25°C, with the exception of the snow-fed lochan in Coire an Lochain (15°C, temperature of inflow from snow patch 10°C), and the small pool in Coire an t-Sneachda (17°C), which was only about 1 m deep instead of its more usual depth of about 2 m (at bottom the latter pool had a temperature of only 12°C).

As regards geology, those waters above 500 m are on granite, while the lower ones lie on the Moine metamorphic series. In the latter case however, there is likely to be considerable variation in the makeup of the overlying drift, with some lakes near the geological boundary probably lying on or draining predominantly granitic materials. Loch Morlich, and to a lesser extent Loch an Eilein, must derive a good deal of drainage from the granite uplands, although lying on top of Moine substrata. Another point to be remarked is that many of the waters come from very peaty surroundings. The three dried-out pools (9, 12, and 16) are the main examples, and of the lochans, Dubh a'Chadha (8), which lies in partly eroding *Trichophorum caespitosum-Calluna vulgaris* moorland.

Another major point of difference is in the type of drainage. Many of the lochans possess neither inflow nor outflow streams, and these are termed seepage lakes. Nos. 5, 8, 10, 13, 14, and 15 appear to belong to this class. Those lakes with inflow and outflow streams are called drainage lakes, though in the case of many of the smaller lochans these streams may be of negligible flow in dry summers.

The three almost dry peaty pools have already been mentioned, but it may be added that they are all in situations of normally stagnant drainage even in wetter seasons. The small artificial drainage lake by Drumintoul Lodge (22) was at the time of sampling also very low, and the water was full of *Nitella;* however, there was still a distinct trickle of water through the breached dam, and the inflow was still running. Moreover, the water was quite clear, in contrast to the strong brown peaty tint to the waters of the aforementioned pools.

Three springs were also sampled, two at high levels on the granite massif, and one on the Moine Series in the Spey valley close by Loch an Eilein. All were very cold, that above Loch Einich being at 4°C, the Marquis Well at 6°C, and the spring by Loch an Eilein at 7°C. The first of these springs marks the beginning of a long series of bryophyte cushions extending down the hillside along the line of flow.

METHODS

Surface waters were collected in pint polythene bottles from the edges of the lakes where there is least vegetation, between July 21 and July 25, 1955. On return to the

TABLE 2. *Dissolved constituents in waters from the Cairn Gorm and Strath Spey areas*

Name	Approximate altitude m	pH Before aeration	pH After aeration	Total cations m. equiv./L	Na	K	Ca	Mg	HCO_3	Cl	SO_4	NO_3-N	SiO_2	Remarks	Map reference (sheet 43)
					parts per million										
On Granite															
1. Spring, Marquis Well, Cairn Gorm	1200	5.3	5.7	0.11	1.6	0.1	0.4	0.2	nil	1.8	1.9	0.08	2.2	Spring flush	006042
2. Snow patch, Coire Cas.	1150	5.8	6.0	0.02	0.3	0.1	nil	nil	0.4	<0.5	<0.5	<0.02	0.8	Clean and coarsely crystalline snow from beneath surface	002041
3. Spring, above Loch Einich	925	5.4	6.0	0.12	1.6	0.2	0.6	0.1	0.5	1.9	2.1	0.06	2.9	Spring flush	925981
4. Lochan, Coire an Lochain, beneath Cairn Lochan	925	5.5	5.9	0.06	0.8	0.2	0.2	0.1	0.2	0.9	0.9	<0.02	1.9	Small drainage lake, still snow-fed, grassy area	981028
5. Lochan, Coire an t-Sneachda	925	6.0	6.5	0.12	1.5	0.2	0.8	0.1	1.0	1.5	2.2	0.05	2.1	Small seepage lake, very rocky	995032
6. Lochan, Coire an Lochain, beneath Cairn Lochan	900	6.1	6.7	0.14	2.2	0.3	0.3	0.2	1.9	2.2	1.5	0.02	6.7	Small drainage lake, peaty area	981030
7. Loch nan Cnapan	850	5.9	6.4	0.14	1.7	0.2	0.9	0.1	0.9	2.3	2.1	<0.02	1.0	Small drainage lake, *Nardus* grassland	917960
8. Lochan Dubh a' Chadha	575	4.6	4.6	0.20	2.8	0.4	0.3	0.4	nil	4.7	2.8	<0.02	0.9	Small seepage lake, in peaty moorland	969066
9. Former lochan about 1 km ESE of Lochan Dubh a' Chadha	550	4.4	4.4	0.92	13.7	2.6	0.8	2.2	nil	23.9	5.0	0.02	1.6	Brown water from quaking *Sphagnum*—sedge bog	977063
10. Lochan t-Seilich	500	5.7	6.2	0.21	2.8	0.3	1.3	0.2	1.1	4.2	3.3	0.04	1.0	Small seepage lake, between scree and eroded bog	915005
11. Loch Einich	500	6.4	6.7	0.17	2.0	0.2	1.3	0.2	1.0	2.7	3.1	0.07	2.2	Large drainage lake	915990
12. Raised bog, by Lochan t-Seilich	500	3.9	3.9	0.78	9.5	0.4	1.2	2.0	nil	14.5	14.6	<0.02	1.0	Brown water from bare peat pool on eroded bog	916005
On Moine Series															
13. Unnamed lochan W of Loch Morlich	350	5.8	6.2	0.20	2.7	0.5	0.7	0.3	1.2	4.4	1.5	0.08	1.0	Small seepage lake on hillside, *Sphagnum* and peat round margin	994095
14. An Lochan Uaine	350	6.7	7.5	0.60	5.2	0.8	5.9	0.9	16.9	7.6	4.9	0.02	1.2	Small rocky seepage lake	001105
15. Lochan nan Nathrach	350	5.4	5.7	0.25	4.2	0.3	0.6	0.3	nil	6.0	2.7	0.06	1.3	Small seepage lake	960085
16. Nearly filled in lochan SW of Loch Morlich	350	4.8	5.0	0.67	7.8	7.3	1.4	0.7	nil	14.6	5.1	0.02	4.5	Brown water from pool on peaty lake mud	953082
17. Lochan nan Geadas	325	6.7	7.2	0.44	5.9	0.4	2.6	0.5	9.1	7.1	3.0	0.03	3.5	Small drainage lake	955088
18. Loch Morlich	325	6.4	6.9	0.25	3.3	0.4	1.4	0.3	4.7	3.8	2.5	<0.02	3.5	Large drainage lake	965094
19. Lochan W of Loch an Eilein	300	6.6	7.3	0.49	6.2	0.6	3.0	0.7	8.2	8.7	3.8	<0.02	2.4	Small drainage lake	917078
20. Spring, near Loch an Eilein	275	6.7	8.1	1.52	12.3	1.8	13.3	3.9	56.7	13.9	7.3	0.03	27.0	Spring in hillside pinewood	902081
21. Loch an Eilein	250	6.5	7.2	0.43	5.4	0.6	2.7	0.5	7.6	7.1	4.0	<0.02	6.3	Large drainage lake	898075
22. Artificial loch at Drumintoul Lodge	250	8.8	8.6	0.97	7.8	1.5	9.5	1.4	32.6	9.0	5.7	0.04	4.5	Small drainage lake, dam breached and very low	922112
23. Lochan N of Loch an Eilein	225	6.8	7.7	0.89	8.7	1.4	6.9	1.6	25.1	10.6	6.1	0.03	1.1	Small drainage lake in grassy birch woods	899094
24. Loch Pityoulish	200	6.9	7.5	0.68	6.5	1.0	5.8	0.9	17.3	8.5	5.8	0.02	4.5	Large drainage lake in grassy birch woods	920135

laboratory within a few days the samples were filtered through washed Whatman 541 papers, the first portion being discarded. Analytical methods were as outlined by Gorham (1955), except that the bicarbonate endpoint was taken as pH 5.7 (the value for distilled water in equilibirum with atmospheric carbon dioxide). Silica was estimated by visual colorimetry using the ammonium molybdate procedure after reduction of the yellow color to molybdenum blue.

RESULTS

The analytical data are given in Table 2, in order of decreasing altitude, with the granite-Moine transition marked by samples 12 and 13. Results are given as parts per million (= milligrams per litre), except for pH and total cations, the latter being given as milli-equivalents per litre.

Total salts

Figure 1 shows a generally good balance between total cations and total anions,

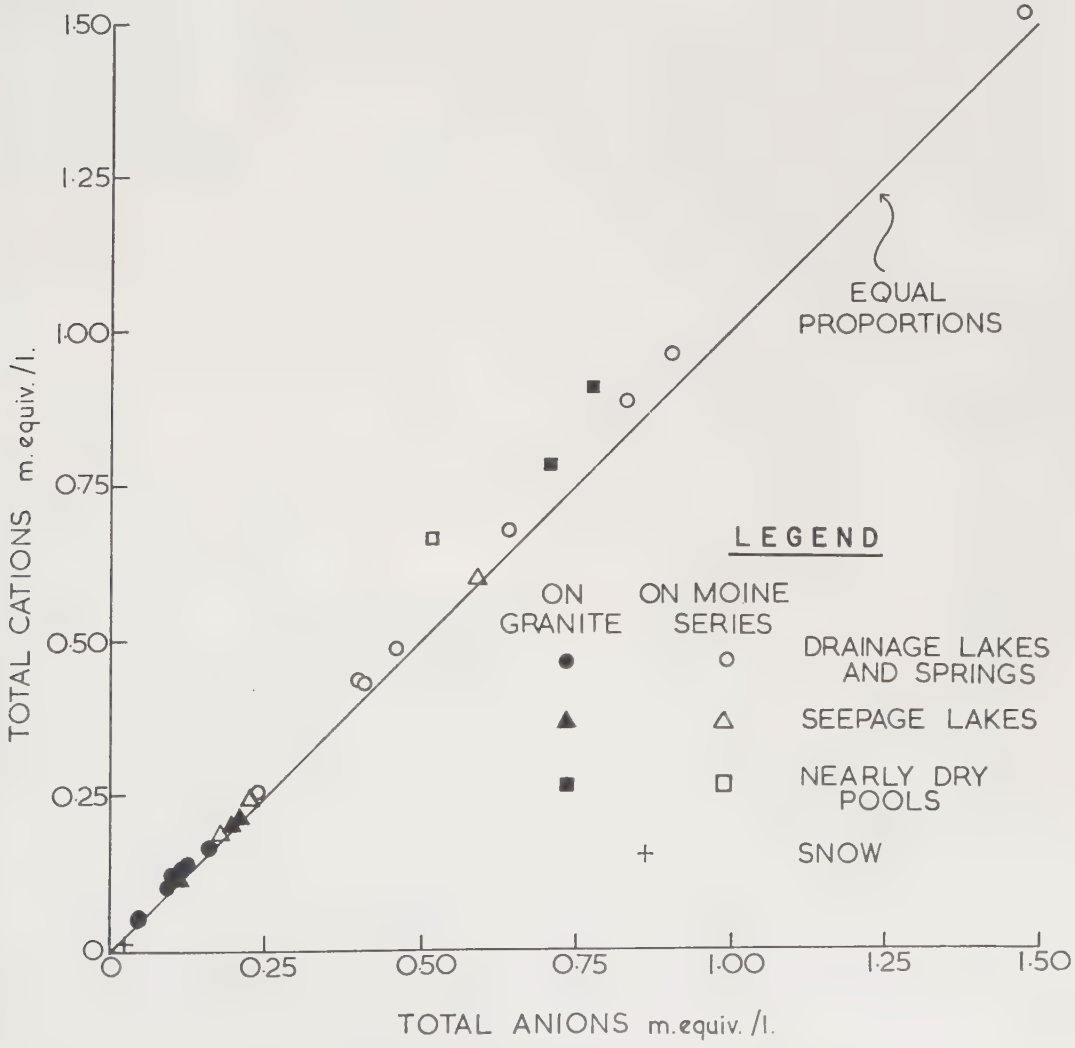

FIG. 1. The balance of cations and anions in the waters.

except in the three almost dry pools (9, 12, and 16), where cations are distinctly in excess of anions. It seems reasonable to suppose that in these very brown and peaty waters there are appreciable amounts of organic acid anions balancing some of the cations. Even in the other waters there is a cation excess of a few per cent, but this is believed to be due to a slight (and for present purposes unimportant) under-estimation of the strong acid anions, as it occurs in the waters from springs and rocky lakes as well as those from peaty sites.

The range of total salt concentration in these waters is rather great, although none of them could be said to be rich in salts. The melted snow, with about 0.02 m.equiv./L, is lowest; while the lowland spring yields the highest concentration of 1.52 m.equiv./L. The upland waters on granite are in general much poorer in salts than the lowland waters on the Moine metamorphic

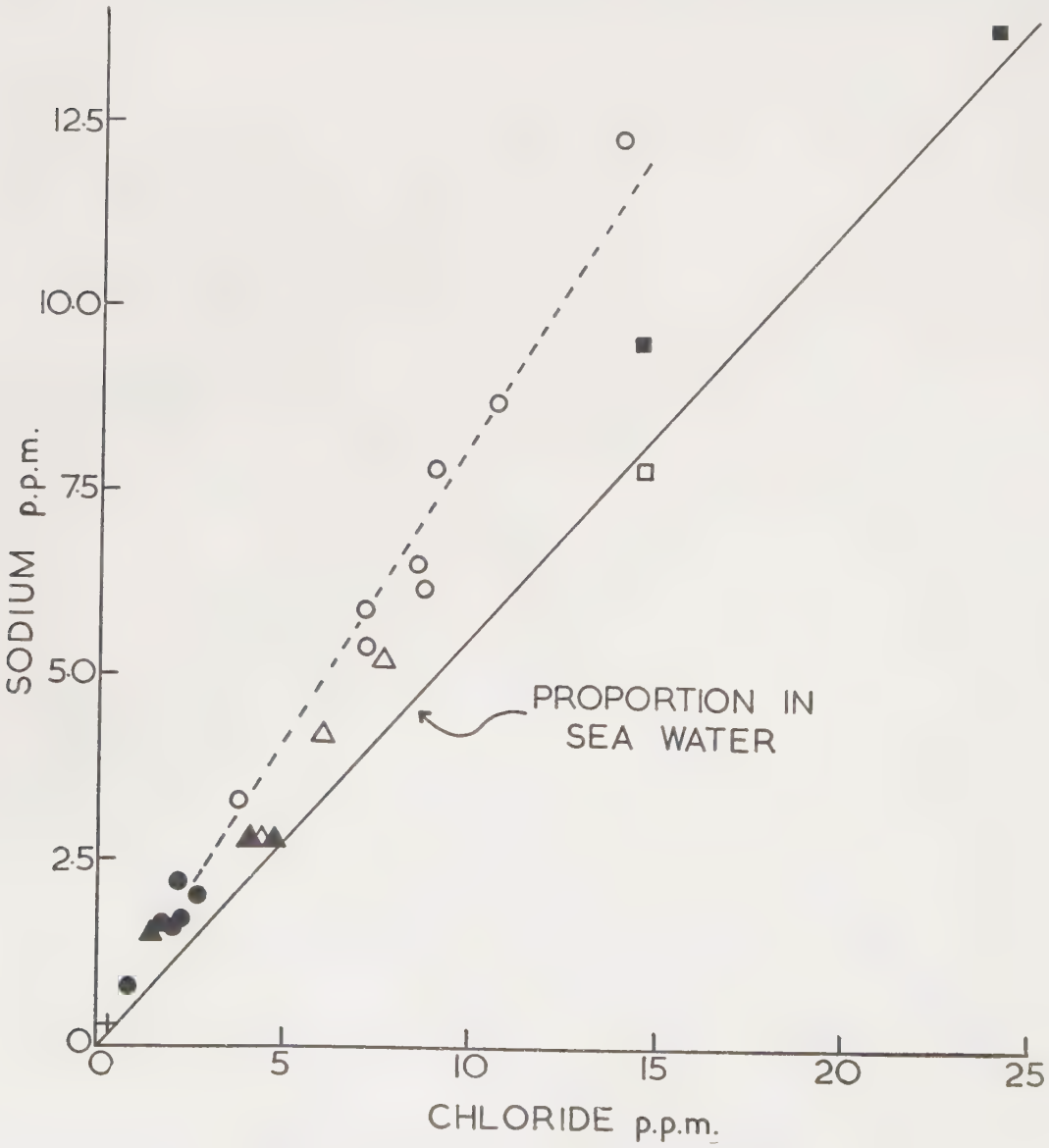

Fig. 2. The relation between sodium and chloride.

rocks, owing partly to greater evaporation at the lower levels, and partly to the weathering of greater amounts of lime from the Moine rocks.

Chloride, sodium, and magnesium

The marked correlation of sodium and magnesium with chloride in these waters, illustrated in Figures 2 and 3, suggests that large proportions of these two cations derive from sea spray, which may be assumed to supply almost all the chloride (cf. Conway 1942, Eriksson 1955). However, the *Na/Cl* ratio in most of the present samples is considerably higher than in sea water, probably owing to some sodium release from weathering soils and rocks, far richer in this element than in chloride. Evidence of the high lake ratio is given in Figure 2, which shows that most of the points lie above the line representing the proportions of sodium and chloride in sea water. It may also be seen from Figure 2 that the drainage lakes (dashed line in Fig. 2) exhibit a greater sodium excess than do the seepage lakes (including the almost dry peaty pools), which in general fall between the two lines in the figure. This might be expected, since the drainage lakes probably receive more moisture by ground-water percolation through mineral soil horizons, and the seepage lakes (and pools) more by runoff over the thick surface humus layers.

In the more dilute waters (chiefly on granite) the *Mg/Cl* ratio is on the average very similar to that in sea water, as appears from Figure 3. This suggests that rather little magnesium is leached from the granitic materials (which probably predominate also in the basins of the more dilute waters on Moine strata, since these are all rather near the geological boundary). Among the

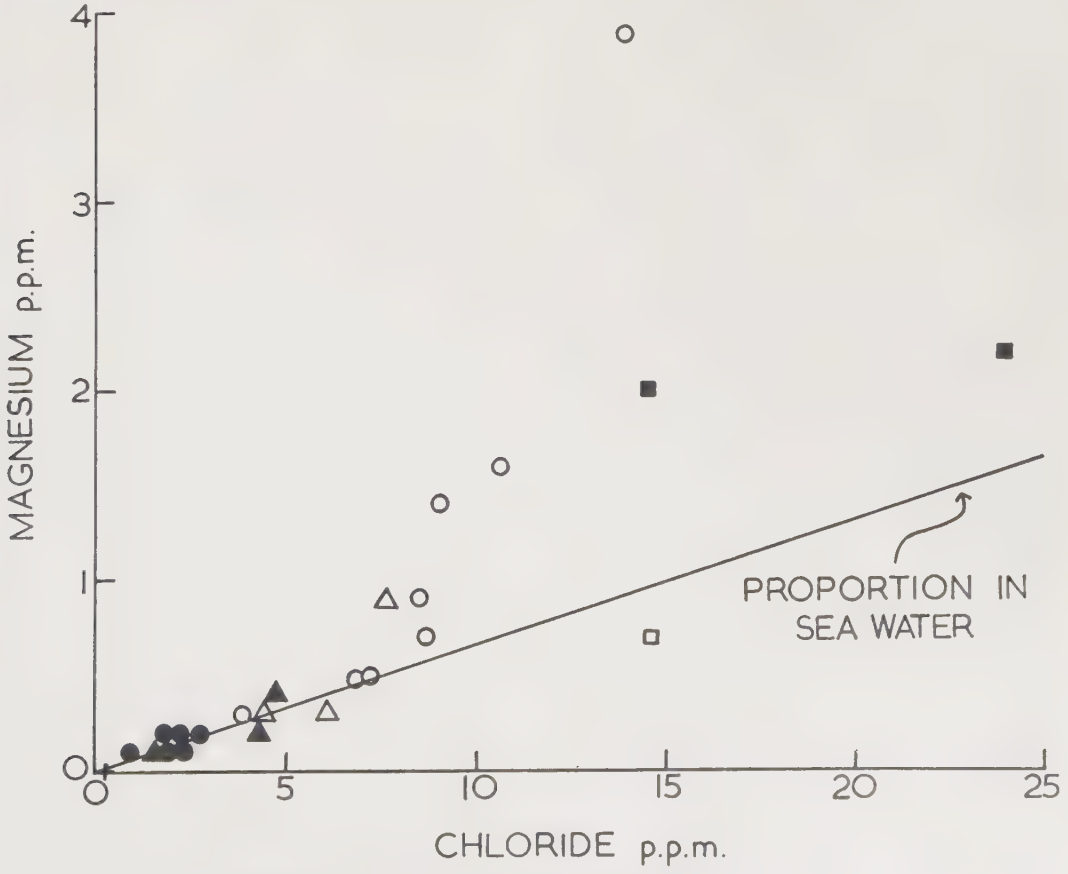

Fig. 3. The relation between magnesium and chloride.

waters on granite the highest *Mg/Cl* ratio comes from the acid pool on an eroding raised bog (12), where it seems likely that magnesium adsorbed on the peat colloids has been displaced into solution by exchange for hydrogen ions, the latter being produced by oxidation of organic sulphur compounds in the peat to form sulphuric acid (cf. Gorham 1956b). In the lowland lakes the situation is different, with a trend toward higher ratios in the more concentrated waters, especially that of the spring. This may be taken to indicate release of magnesium by weathering of the Moine rocks and their derived soils.

A most noteworthy feature of the present results is that chloride, supplied almost entirely by atmospheric precipitation, increases greatly in concentration at the lower altitudes. From about 2 ppm at 900 m (only 0.9 ppm in the lake still fed by snow melt) this ion rises at an increasing rate to 9–10 ppm in the lakes between 200 and 300 m above sea level, and even greater concentrations are recorded from the three pools which had almost dried up. Results of this kind clearly point to evaporation as a factor of extreme importance in determining the salt concentrations of waters in this area, especially in periods of such abnormally hot dry weather. Moreover, at a given altitude the smaller lakes tend to show the higher chloride concentrations. For instance, on Moine strata the four small drainage lakes (17, 19, 22, 23) may be compared with the three large ones (18, 21, 24). In altitude the former average about 275 m, the latter 255 m, so that on the basis of altitude alone the smaller lakes might be expected to be if anything a trifle more dilute. In fact, the small lochans are almost 40 per cent richer in chloride, averaging 8.9 ppm as against 6.5 ppm in the large lochs. This difference reflects not only the greater *area/volume* ratio of small lakes, leading to a proportionally high degree of evaporation, but also the fact that the larger lakes receive a good deal of their water from levels far above them, where it has not been so subject to evaporation in the gathering ground. An additional factor to be considered here is the greater vegetation cover at lower levels, which through transpiration may increase evaporation. Many of the smaller lowland lochans in particular lie in heavily vegetated drainage basins.

Calcium and bicarbonate

Calcium and bicarbonate ions are low in the waters on granite, the maximum concentrations being 1.3 and 1.9 ppm, respectively. The range of calcium concentrations, 0.2–1.3 ppm, is about the same as in pools on raised and blanket bogs dependent for their mineral supply solely upon atmospheric precipitation (Gorham 1956a, 1956b, and unpublished data; Gorham and Pearsall 1956). It thus appears that the granite releases its calcium (in general about 0.5–2.0 per cent by weight) only very slowly. The waters on Moine rocks reach considerably higher levels of calcium concentration, ranging from 0.6 to 13.3 ppm, the latter value being recorded from the spring near Loch an Eilein. While greater evaporation at the lower elevations of the lakes on the Moine Series is partly responsible for the higher calcium concentrations there, this is not the only factor involved, since the ratio of calcium to chloride is also distinctly above average in the richer Moine lakes. The correlation between bicarbonate and calcium, present in these waters in approximately equivalent amounts (see Fig. 4), indicates rock and soil weathering as the source of most of this calcium. Very little can be supplied from sea spray, as calcium is very low in sea water relative to chloride. Although the Moine rocks do not in general contain a great deal of calcium (as judged by analyses from northern Scotland compiled by Guppy 1931), they are perhaps somewhat richer than the granites. There are, in addition, occasional intrusions of rocks such as epidiorites and hornblende schists, which are often quite rich in calcium; and moreover the ordinary mica-schists of the Moine Series may well be more easily weathered than the granites, and so release their calcium more rapidly.

The two lakes on Moine strata which are lowest in calcium (13 and 15, with 0.7 and 0.6 ppm) are both seepage lakes in pine-heather moorland. Presumably they depend largely on surface runoff, or percolation

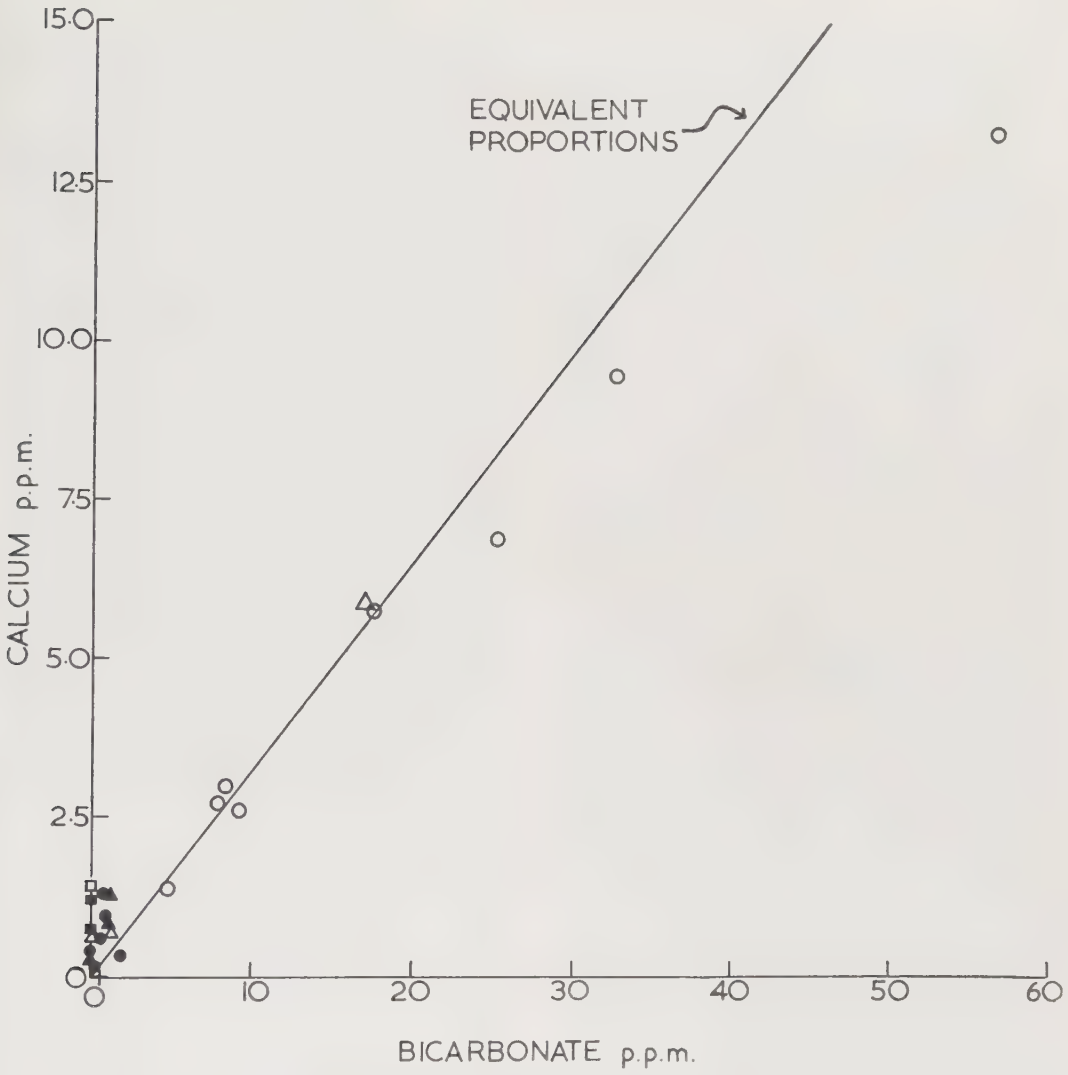

Fig. 4. The relation between calcium and bicarbonate.

through the leached horizons of the sandy podzols, while the drainage lakes receive some contribution from deeper subsoil waters. However, An Lochan Uaine (14), also a seepage lake, is much richer in calcium (5.9 ppm). In this connexion it may be pointed out that Watt and Jones (1948) suggest the presence of limestone in the vicinity; and it may also be significant that this lochan drains a bare rocky hillside, whose mineral debris is not much isolated from surface runoff by a layer of peaty *mor* humus as in the case of the other two lochans. Of the Moine drainage lakes Loch Morlich (18) is lowest in calcium, with 1.4 ppm, owing probably to its reception of much water from gathering grounds high up in the granite hills.

Acidity

Acidity varies considerably in these waters, with pH ranging from 3.9 in the sample from a raised-bog pool to 8.8 in the partly drained artificial lake by Drumintoul Lodge. In the three most acid samples (8, 9, and 12) there is a marked correlation between the concentrations of hydrogen ions and sulphate, from which it may be inferred that the high acidity is due to the presence of free sulphuric acid (cf. Gorham

1956b). The rather extreme alkaline pH of the Drumintoul water (22) before aeration is probably owing to exhaustion of most of the dissolved carbon dioxide present by the very dense growths of *Nitella*. That under natural conditions carbon dioxide contributes greatly to the hydrogen ion concentration of many of the less acid waters is shown by the marked increase in pH following aeration. After aeration, pH is found to be closely related to bicarbonate concentration, and hence to the amount of lime weathered from the rocks and soils. Above pH 7 (after aeration) bicarbonate concentrations rise sharply.

Only the small lochans reach high levels of acidity. The big lochs exhibit a pH range of 6.4–6.9 before and 6.7–7.5 after aeration.

Potassium

The ratio of potassium to chloride in these waters is considerably above that in sea water, suggesting that this element is derived more from weathering of rock and soil minerals than from sea spray. However, the low concentrations in the upland waters are very similar to those in rain (Gorham 1955; Emanuelsson, Eriksson, and Egnér 1954), so that much of the potassium probably comes from blown dust. The slightly higher K/Cl ratios in the richer lakes on Moine substrata point to a greater release of potassium from these rocks.

The two highest potassium concentrations, 7.3 and 2.6 ppm, come from the almost dried-up pools (16 and 9, respectively). The first figure represents a site still showing some areas of bare mud bottom, now being colonized by *Carex rostrata* and *Menyanthes trifoliata*, with occasional *Sphagnum* hummocks. This sample also shows an extremely high K/Cl ratio. The second figure in contrast represents a site where the former lake bottom has become completely covered by a quaking bog mat of *Eriophorum angustifolium*, *Carex rostrata*, and *Sphagnum papillosum* hummocks; and here the K/Cl ratio is not abnormal. Presumably the large quantity of potassium in sample 16 has been mainly released from decaying organic materials in the dried-out lake bottom, where at present there is not an abundance of organisms to absorb it from the remaining water.

Sulphate

This ion may, like chloride, be assumed to come almost wholly from atmospheric precipitation, since rocks are generally very low in sulphur (cf. Conway 1942, Gorham 1955). Again like chloride, its concentration in these surface waters increases greatly with decreasing altitude. However, it is of much interest that the increase is not by any means proportional to the rise in chloride, so that the SO_4/Cl ratio is much less in the Spey valley than on the Cairn Gorm hills. For example, the lakes on the lowland Moine strata give a SO_4/Cl range of about 0.3–0.7, while for the lakes on the upland granites the range is about 0.6–1.5. Since it seems most improbable that the granites are supplying appreciably large amounts of sulphur to the upland lochans (sufficient to increase the SO_4/Cl ratio well above that in the lowlands), some other explanation of this phenomenon must be sought. In the absence of concrete evidence, the following conjecture may perhaps serve at least as a basis for further study.

A notable feature of the series of waters under investigation is that the lakes on Moine rocks lie below the tree line, while all the granite lakes are well above it. This fact may assume great importance when taken in conjunction with the studies of Eriksson (1955) and Tamm (1953), which strongly suggest that vegetation may capture giant sea salt particles from the atmosphere, these being subsequently washed into the streams and lakes during rainy weather. If such sea salt capture is an important source of chloride in natural waters, then lakes below the tree line might be expected to receive much more than those above it. However, in the present instance it is necessary to assume further that sulphate is not subject to the same degree of vegetation capture as chloride. This seems not unreasonable, as a good deal of the sulphur in the air is probably present in gaseous form, whereas the chloride must be largely derived from solid sea salt particles. An additional point of interest here is that, at comparable altitudes in both the upland

and lowland groups of waters, lakes draining the more rocky areas (*e.g.* 4, 5, 11, 14, 18, 21, 22, 24) tend to exhibit higher SO_4/Cl ratios than those in well-vegetated sites (*e.g.* 6, 8, 13, 16, 17, 19). The lochan in Coire an t-Sneachda (5), with clearly the most rocky surroundings of all, yields the maximum SO_4/Cl ratio of 1.5. As would be expected on this basis, the larger lakes tend to exhibit ratios on the high side, since they all drain fairly extensive areas of relatively open ground. Among the smaller lakes, no. 13 probably has the most heavily vegetated surroundings, and gives the minimum SO_4/Cl ratio for all the lakes, 0.3.

The two almost dry pools on granite (9 and 12) show a marked difference in SO_4/Cl ratio. The latter, a pool on the bare peat surface of an eroded raised bog, yields a rather high ratio of 1.0, probably due to release of sulphur from the decaying peat. The former, in contrast, gives the lowest ratio of all, about 0.2. In this site however, there is a complete cover of vigorously growing *Sphagnum* and *Carex*, and on pressing the sampling bottle into the spongy surface to obtain water, a distinct smell of hydrogen sulphide was noticed. In this instance then, reduction of sulphate may be inferred as bringing about the abnormally low SO_4/Cl ratio.

Nitrate and phosphate

Nitrate nitrogen is low in all these waters (at least in summer), never rising to 0.1 ppm in the present samples. Dissolved phosphate must be extremely scarce, since it was undetectable (<0.001 ppm) in all cases examined (2, 12, and 16 not tested).

Silica

The snow patch melt-water shows the lowest value for this constituent, as might be anticipated, with 0.8 ppm SiO_2. The lowland spring gives the highest concentration, 27 ppm. The lakes range from 1 to 7 ppm, with most of them near the lower limit; and only a few drainage lakes with pH values above 6 possess waters with more than 2 ppm SiO_2.

DISCUSSION

From the foregoing results it is evident that the amounts of dissolved materials in the waters of the Cairn Gorm-Strath Spey area are influenced by several environmental factors. The climatic factor of evaporation is one of the most important, but is itself mainly dependent in this instance upon the nature of the relief, as determined originally by the way in which the Cairn Gorm plateau was dissected by the Spey River, and subsequently by the processes of glaciation. A more independent aspect of climate is however to be seen in the exceptionally warm dry summer conditions of 1955, which accentuated the significance of the evaporation factor.

The influence of topography is apparent in a variety of ways. As has been mentioned, both altitude and basin shape affect evaporation from a lake. In addition, the influence of relief upon persistence of snow may affect the water supply to many lochans (*e.g.*, the lochan in Coire an Lochain). Its effect upon drainage, which may be discerned not only in the very locations of the lakes in the glacial deposits but also in the contrast between drainage and seepage lakes, is necessarily of great importance, as is its effect upon peat accumulation. Lastly topography—partly through its effects upon local climate—has a good deal of influence upon the distribution of vegetation and the level of the tree-line, and hence in yet another way upon evaporation, and probably also upon the balance between atmospheric chloride and sulphate supply.

The underlying geology, besides being ultimately concerned in defining the patterns of relief, plays a large part in determining the amounts of bases reaching these natural waters. This may be seen for example by comparing the ratios of bicarbonate to chloride or sulphate in drainage lakes on granite with those in such lakes on Moine substrata.

The biological factor remains to be considered. In its dependent aspect (with plant distribution conditioned by climate, topography, and geology) it affects evaporation, and probably the supply of atmospheric salts. The sulphur balance may also be influenced by oxidation and reduction processes in peaty soils, and of course the concentrations of such biologically important substances as nitrate, phosphate,

and silica fluctuate markedly in relation to biological demand. Also to be remarked is the likelihood that man has had some influence (through deforestation, grazing, and atmospheric pollution) upon the composition of these waters.

A final feature of the present study deserving of emphasis concerns the importance of atmospheric precipitation in supplying dissolved ions to these waters. Comparison of the ionic concentrations in lake waters on granite with those of rain samples collected in the English Lake District (Gorham 1955) reveals considerable similarity in ionic concentrations, while consideration of analyses from both the granite and Moine lakes leads to the conclusion that the most evident effect of soil percolation upon rain is the addition of calcium bicarbonate released through weathering of the rock and soil minerals.

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NOTES AND COMMENT

Simplified Computations for the Dynamic Height Method of Current Determination in Lakes[1]

INTRODUCTION

A dynamic height method for the determination of current directions and velocities in deep lakes has been developed by Ayers (1956). This method involves very considerable amounts of arithmetical calculation and hence contains numerous possibilities for arithmetical error. Successful reduction of the amount of computation required would achieve both economy of time and reduced chances for error. This paper presents a successful simplified computation routine for the freshwater dynamic height method.

The simplified computation routine consists of a more convenient rearrangement of the original computation method and in no way changes the theoretical considerations upon which that method was based. The reader is referred to Ayers (1956) for the full development of theory and the original method.

Formal thanks are tendered to the University of Michigan Biological Station, where much of this work was carried out.

THE SIMPLIFIED METHOD

The new method differs from the original in two ways: 1) the expansive effects of temperature and the compressive effects of pressure are independently determined and applied, and 2) new tables directly readable to 0.1°C have been prepared. Independent application of the thermal and pressure effects eliminates the laborious double interpolations commonly required in the original method.

The new method first determines the specific volume anomaly at observed temperature and zero hydrostatic pressure ($10^5\ \delta_{t,o}$), then applies a compression correction ($\Delta a_{t,p}$) at observed temperature and *in situ* pressure to obtain the anomaly at temperature and pressure. The new specific volume anomaly table (Table 1) was prepared by graphing the zero-pressure portions of Ayers' (*loc. cit.*, Table 2) original anomaly table and reading anomaly values ($10^5\ \delta_{t,o}$) at each 0.1°C. The new table of coefficients of compression (Table 2) was prepared by graphing and reading at each 0.1°C Ayers' (*loc. cit.*, Table 1) compressibility coefficients. As in the original paper, the coefficient of compressibility (in cm^3/cm^3/atmosphere) is numerically equal to compression in cm^3/atmosphere (C_t) when applied to a volume of one cubic centimeter. The pressure effects are handled, as in the old method, by the decibar system: pressure in atmospheres, p = depth in meters ÷ 10 meters/atmosphere. As before, $10^5 C_t \times p = \Delta a_{t,p}$ and $10^5\ \delta_{t,o} - \Delta a_{t,p} = 10^5\ \delta_{t,p}$, the required anomaly at temperature and pressure.

As in the old method, the resultant anomaly ($10^5\ \delta_{t,p}$) is required for the surface temperature, for each subsurface inflection of the temperature curve, and for the temperature at the reference level.

COMPARISONS OF THE NEW AND OLD METHODS

As indicated above, the required resultant anomaly for any subsurface temperature and pressure can be obtained (in the new method) by two table readings, one division, one multiplication, and one subtraction—a total of five operations. Under the old method obtaining a single resultant anomaly at temperature and pressure involved a total of thirteen operations as follows:

[1] Contribution No. 3 from the Great Lakes Research Institute, University of Michigan, and Contribution No. 883 from the Woods Hole Oceanographic Institution.

TABLE 1. *Specific volume anomaly* ($10^5\ \delta_{t,o}$)

°C	0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	13.0	12.4	11.5	10.8	10.2	9.6	9.0	8.4	7.9	7.4
1	7.0	6.6	6.2	5.7	5.3	4.9	4.6	4.2	3.8	3.5
2	3.0	2.8	2.6	2.3	2.0	1.8	1.6	1.4	1.2	1.1
3	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
4	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
5	1.0	1.1	1.2	1.3	1.5	1.7	1.9	2.1	2.4	2.7
6	3.0	3.3	3.7	4.1	4.5	4.9	5.3	5.7	6.1	6.5
7	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5
8	12.0	12.6	13.2	13.9	14.6	15.3	16.0	16.7	17.4	18.3
9	19.0	19.7	20.4	21.2	22.0	22.8	23.6	24.4	25.3	26.1
10	27.0	28.0	29.0	30.0	31.0	32.0	33.0	34.0	35.0	36.0
11	37.0	38.0	39.1	40.2	41.3	42.5	43.6	44.7	45.8	46.9
12	48.0	49.1	50.2	51.4	52.6	53.8	55.0	56.2	57.4	58.7
13	60.0	61.3	62.6	63.9	65.2	66.5	67.8	69.1	70.4	71.7
14	73.0	74.4	75.8	77.2	78.6	80.0	81.4	82.8	84.2	85.6
15	87.0	88.6	90.2	91.8	93.4	95.0	96.6	98.2	99.8	101.4
16	103.0	104.7	106.4	108.1	109.8	111.5	113.2	114.9	116.6	118.3
17	120.0	121.8	123.6	125.4	127.2	129.0	130.8	132.6	134.4	136.2
18	138.0	139.9	141.8	143.7	145.6	147.5	149.4	151.3	153.2	155.1
19	157.0	159.0	161.0	163.0	165.0	167.0	169.0	171.0	173.0	175.0
20	177.0	179.1	181.2	183.3	185.4	187.5	189.6	191.7	193.8	195.9
21	198.0	200.3	202.6	204.9	207.2	209.5	211.8	214.1	216.4	218.7
22	221.0	223.3	225.6	227.9	230.2	232.5	234.8	237.1	239.4	241.7
23	244.0	246.4	248.8	251.2	253.6	256.0	258.4	260.8	263.2	265.6
24	268.0	270.6	273.2	275.8	278.4	281.0	283.6	286.2	288.8	291.4

TABLE 2. *Coefficients of compression* ($10^5\ C_t$)

°C	0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	5.250	5.243	5.236	5.230	5.224	5.219	5.214	5.209	5.204	5.200
1	.195	.191	.187	.183	.179	.176	.173	.170	.167	.163
2	.160	.157	.154	.151	.148	.145	.142	.139	.136	.133
3	.130	.128	.125	.122	.120	.118	.115	.112	.110	.108
4	.105	.103	.100	.098	.095	.092	.089	.087	.085	.082
5	.080	.078	.076	.074	.072	.070	.068	.066	.064	.062
6	.060	.058	.057	.055	.053	.051	.049	.047	.045	.044
7	.043	.041	.039	.038	.036	.034	.033	.031	.029	.028
8	.027	.025	.024	.023	.022	.020	.019	.018	.016	.014
9	.013	.012	.011	.010	.008	.007	.006	.004	.003	.001
10	5.000	4.998	4.997	4.996	4.994	4.993	4.992	4.990	4.989	4.987
11	4.985	.984	.983	.982	.981	.980	.979	.978	.977	.976
12	.975	.974	.973	.971	.970	.969	.968	.967	.966	.965
13	.964	.962	.961	.960	.959	.958	.957	.956	.955	.954
14	.953	.953	.952	.951	.950	.949	.948	.947	.946	.945
15	.944	.943	.942	.941	.941	.940	.939	.938	.938	.937
16	.936	.936	.935	.934	.933	.932	.932	.931	.931	.930
17	.929	.929	.928	.928	.927	.926	.925	.924	.923	.923
18	.922	.921	.921	.920	.919	.919	.918	.918	.917	.916
19	.916	.915	.914	.914	.913	.913	.912	.912	.911	.911
20	.910	.910	.909	.909	.908	.908	.907	.907	.906	.906
21	.905	.905	.904	.904	.903	.903	.903	.903	.902	.902
22	.902	.902	.901	.901	.901	.901	.900	.900	.899	.899
23	.898	.898	.898	.897	.897	.896	.896	.895	.894	.894
24	.893									

a. Reading anomalies at temperatures adjacent to the observed temperature and at pressure less than observed pressure (2 table readings)

b. Interpolating to anomaly at observed temperature and less than observed pressure (1 subtraction, 1 multiplication, and 1 addition)

c. Reading anomalies at temperatures adjacent to observed temperature and at pressure greater than observed pressure (2 table readings)

d. Interpolating to anomaly at observed temperature and greater than observed pressure (3 operations as in b above)

e. Interpolating between anomalies at observed temperature (items b and d above) for anomaly at observed temperature *and* pressure (3 operations as in b above).

The new computation reduces to less than half the number of operations (and the time, labor, and chance for error) involved in obtaining each individual resultant specific volume anomaly.

Subsequent computations leading to the final dynamic height at each station are carried out as in the original paper and are no less time-consuming and laborious than before. They do benefit, however, by the lesser chance for error inherent in the new method.

As a comparison of the accuracy of the two methods, the dynamic heights of 56 stations taken in Lake Michigan on 28 June 1955 have been worked out by each method. While there were minor variations in the insignificant terminal decimal places, the final dynamic heights rounded off to the same values by either method. There is, then, no loss of accuracy resulting from the simpler new method. The total labor (with calculator) required for the two determinations of this topography was 31 man-hours by the original method and 13.5 man-hours by the new.

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Cordylophora lacustris Allman from Chagrin Harbor, Ohio[1]

Frequently it has been stated (or assumed) that the cnidarian *Cordylophora lacustris* Allman is a brackish-water animal which in its evolution is in the process of migrating into fresh water (*e.g.*, see Carpenter 1928). Specimens sometimes are abundant in suitable brackish-water locations (Wesenberg-Lund 1939), and occasionally they are found in fresh water as well. Reports of collections from fresh water in Europe are not frequent, but neither are they exceptionally unusual. Freshwater collections also have been made in Asia, Australasia, and Africa. In the Western Hemisphere publications listing the occurrence of the species in fresh water have been infrequent, and even some of these records are only doubtfully nonmarine. Thus, Blake (1932) collected specimens in the Charles River, Massachusetts, where, however, the chlorinity was "equivalent to 0.224% NaCl"; Clarke (1878) found specimens attached to *Potamogeton* and *Nitella* in Curtis Creek, Baltimore, Maryland, where the water was fresh at times, but was variable in saltiness according to the stage of the tide; Bibbins (1892) listed this and other similar localities in Chesapeake estuaries as habitats for the species; Hargitt (1908) and Nutting (1901) reported the species from freshwater ponds in Massachusetts (Woods Hole, Falmouth, Marthas Vineyard), but these ponds were located so near to the sea that it is doubtful they were strictly fresh from the ecological point of view, even though at the time of collection they may have been so fresh that they did not taste distinctly salty; similarly, Gaggero (1923, 1935) found *Cordylophora* in lagoons of Río de la Plata, Argentina, where the water was fresh, but was affected by the tides, and therefore possibly was somewhat brackish at times (see Roch 1924 for a discussion of this species in relation to salt content of the water).

To my knowledge, the only previously published records in the Western Hemisphere from water that was incontestably fresh are those of Fraser (1944) from the Gatun Locks, Panama Canal; Garman (1922) from Benson Creek near Frankfort, Kentucky; Leidy (1870) and Potts (1884) respectively in the Schuylkill River at Fairmount, and in the Fairmount Reservoir, Philadelphia, Pennsylvania; Smith (1910) from the Illinois River (Quiver Lake) near Havana, Illinois; and Smith (1918) from the Mississippi River (Granite City and East Saint Louis, Illinois), from the Arkansas River at Little Rock, and from the Red River at Shreveport, Louisiana.

In addition, Cope (1883) published a description of a hydroid (?) polyp from Upper Klamath Lake, Oregon, which he named *Rhizohydra flavitincta*. His description was obscure, but Clarke (1884) referred to it as an "imperfectly known form, allied to *Cordylophora*." In some ways Cope's description does appear to fit this genus: "Its coenobium is a mass of creeping yellowish stems," but the stems were "imbedded in a sarcode," and the polyps had only six rays (tentacles?).

A limited number of specimens of *Cordylophora lacustris* Allman were collected on November 4, 1956 near the mouth of the Chagrin River (Chagrin Harbor), Lake County, Ohio by Mr. Lawrence Soffer, a student at Western Reserve University. Some tubes bore no polyps, perhaps because of the lateness of the season. No reproductive polyps were observed. The hydrocauli were attached to a small twig, and were mixed in with the tubes of the much more abundant bryozoan, *Plumatella repens*. There was in addition a rich and varied fauna of vorticellids (*Epistylis*, *Carchesium*, *Vorticella*, etc.). Shortly after the collection date the first severe cold spell of the season set in, and no further specimens could be found. The preserved specimens have been deposited in the U. S. National Museum, catalogue No. 50546.

The present find constitutes the first record for the Great Lakes drainage area, and the first freshwater record for 35 years (since Garman 1922) from North America. Such a history of collection in fresh water gives little indication that the species has as

[1] Sincere thanks are expressed to Dr. Libbie H. Hyman for her valuable suggestions regarding literature sources.

yet successfully established itself in fresh water in North America (although it is possible that thorough search for it in suitable environments would show it to be more common and more widely distributed than available records would lead one to believe). This judgement is strongly supported by the well-known facts (*e.g.*, see Pennak 1953, and Wesenberg-Lund 1939) that freshwater colonies average much smaller than brackish-water colonies, and that they produce fewer gonophores, with decidedly smaller numbers of ova in each gonophore. *C. lacustris* is apparently an extremely euryhaline animal, tolerant, at least for a time, of fresh water. Normally, however, it flourishes only in brackish water locations.

The means by which *Cordylophora* has invaded fresh water are assumed to be by way of the hulls of ships and on the legs of birds. However, so far as the author is aware, no definitive evidence has been published proving either of these means. Ship traffic from salt water to the Great Lakes is frequent and increasing. Chagrin Harbor itself, on the other hand, is accessible from Lake Erie only by small boats, few or none of which ever ply between the distant salt water and Lake Erie. That the species is not always transported by ships is shown by Garnett (1953), who encountered it in an English pond "not directly connected with any water system," and by Rzóska (1949), whose specimens came from the Upper White Nile, 1500–1600 airline miles from the Nile Delta, and far above the upper limits of navigation. Furthermore, Roch (1924) showed that specimens (from brackish water?) were killed when immersed either in full-strength sea water or in Berlin tap water, although they lived in tap water to which a small amount of salt had been added. This would suggest that animals growing attached to ship hulls would succumb to the shock of sudden entry into fresh water. This matter, however, deserves much further study before any final conclusions can be reached.

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Oceanic observations of the Pacific: 1949. Univ. of California Press, Berkeley and Los Angeles, 1957. Editors: Norris W. Rakestraw, Paul L. Horrer, and Warren S. Wooster. Pp. 363 $4.50

Serial observations of temperatnre, saliuity, and chemical constituents of sea water in the Atlantic Ocean for 1908 and subsequent years have been published in *Bulletin Hydrographique* by Conseil Permanent International pour l'Exploration de la Mer, Copenhagen. Publication has normally been annual with a lag of one to four years, but World War I and World War II both produced delayed volumes covering several years.

The Pacific Ocean counterpart has now been initiated with the publication of *Oceanic Observations of the Pacific* for 1949, prepared at Scripps Institution of Oceanography of the University of California, La Jolla. This well-arranged volume presents tabulated data for stations made by (1) Scripps Institution of Oceanography, (2) U. S. Navy Electronics Laboratory, San Diego, and (3) Pacific Oceanic Fishery Investigations, Honolulu.

The preliminary 14 pages include noteworthy features. The Description of Observations from each contributing agency gives not only a brief description of special techniques of measurement and reduction but also a list of published papers pertaining to the 1949 cruises. As in the *Bulletin*, there are charts showing station positions. Four additional charts (North Pacific, South Pacific, and Indian oceans, and antarctic waters south of 50°S) show the total number of bathythermograms in each 5-degree quadrangle for the period 1941–1949.

The tabulations are exceptionally legible, and all material for each station is together on one page, without exception. The stations for each cruise follow one another consecutively, a more convenient arrangement, this reviewer agrees, than is used in the *Bulletin* (where chemical data are in a section separate from temperature and salinity, and where the stations are arranged in a geographical sequence). For each station there are two tabulations. The first presents temperature (C), salinity, oxygen (ml/l), and phosphate phosphorus (μg-at/l) at observed depths, as in the *Bulletin*. The second tabulation, at standard depths or pressures (0, 10, 20, 30, 50, 75, 100, 150, 200, 250, 300, 400, . . . 800, 1000, 1200, 1500, 2000, . . . m or db), with no counterpart in the *Bulletin*, presents interpolated values for the same four quantities and calculated values for three more: sigma-t, specific-volume anomaly, and the anomaly of geopotential thickness of the water column extending up to the sea surface. (There is an omission on page 14, where each tabular column is explained with the exception of PO_4-P.)

Vast numbers of bathythermograms have been collected and filed since 1941, but regrettably few raw observations or digested results have been published. The BT charts in *Oceanic Observations* and the short list of BT dates and positions in the back of the *Bulletin* for 1952 (United States and others are not listed) give slight attention to this valuable material. It occurs to this reviewer that a desirable small step would be to augment the supplementary information for each station by stating the BT depth attained and, if helpful, the identifying bathythermogram number.

Oceanographers increase their difficulties by using different but equivalent quantities or units. It is gratifying to find that *Oceanic Observations* conforms with the *Bulletin* in using salinity rather than chlorinity and in expressing dissolved oxygen in ml(NTP)/l(20 C) rather than in mg-at/l(20 C). On the other hand, *Oceanic Observations* follows the clumsy habit of tabulating two inconsistent quantities: sigma-t (dimensions of density) and specific-volume anomaly. One of *Oceanic Observations'* editors and this reviewer have urged (*Deep-Sea Res.*, **2**: 67, 1954) that sigma-to be replaced with thermosteric anomaly in routine use, so that oceanographers could enjoy the convenience of working with quantities having uniform dimensions of specific volume. Of course the 1949 data were reduced and tabulated years ago, and this labor could not easily be repeated. It is hoped that the proposed simpler scheme will soon be adopted.

It may be noted that conditions at observed depths are tabulated in five columns. The interpolated and computed conditions, which of course contain no information not obtainable from the observed conditions, occupy eight columns; and the number of standard depths exceeds the number of observed depths. Although some readers may use values at standard depths, others will use observed conditions only and will prefer their own interpolation. If it should be desirable to expedite and economize future volumes, the tabulation at standard depths could be omitted.

However, if computed values can feasibly be included, this reviewer would prefer to see them tabulated at observed depths rather than at standard depths, so that the errors of interpolation would be avoided. The desirable variables are thermosteric anomaly, specific-volume anomaly, potential temperature, and potential specific-volume anomaly.

The preface states that hitherto unpublished observations made prior to 1949 will appear in a later volume of *Oceanic Observations*. Data for each succeeding year will appear in individual volumes. The invitation is extended for other agencies engaged in oceanography in the Pacific to utilize this serial for publication.

The University of California is performing a vital service to oceanography in undertaking the publication of Pacific observations and deserves high praise for the excellence of this initial volume.

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Erratum in Vol. 1 of Limnology and Oceanography

p. 305. Line 14 in the left-hand column should read "measuring 0.01 to 0.02 inch across the disk, has"